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(54) Title: NOVEL METHOD FOR IDENTIFYING ANTIBACTERIAL COMPOUNDS (57) Abstract The present invention relates to a method for identifying an antagonist or inhibitor of the expression of a gene encoding a polypeptide essential for bacterial growth or survival as well as for an antagonist or inhibitor of said polypeptide. The invention further relates to a method for improved antagonists or inhibitors. The invention also provides an antagonist or inhibitor of the activity of said polypeptide. The invention is further related to a method for producing a therapeutic agent in a composition comprising said antagonist or inhibitor. Furthermore, the invention is related to the use of the polypeptide and the antagonist or inhibitor as well as to a method to identify a surrogate marker.		

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Novel method for identifying antibacterial compounds

The present invention relates to a method for identifying an antagonist or inhibitor of the expression of a gene encoding a polypeptide essential for bacterial growth or survival as well as for an antagonist or inhibitor of said polypeptide. The invention further relates to a method for improved antagonists or inhibitors. The invention also provides an antagonist or inhibitor of the activity of said polypeptide. The invention is further related to a method for producing a composition comprising said antagonist or inhibitor. Furthermore, the invention is related to the use of the polypeptide and the antagonist or inhibitor as well as to a method to identify a surrogate marker.

Several documents are cited throughout the text of this specification. Each of the documents cited herein (including any manufacturer's specifications, instructions, etc.) are hereby incorporated by reference; however, there is no admission that any document cited is indeed prior art of the present invention.

Since the beginning of the 1980s, a new trend has been observed in the industrialized countries. On the one hand, resistances to antibiotics have increased, which make it difficult or even impossible to treat many of the disease-causing agents. On the other hand, new infectious diseases, which had been unknown up to now, arise, and old diseases return. For example, diphtheria and tuberculosis are old epidemics and increasingly surmounting in many different parts of the world. Especially tuberculosis (TB), a chronic infectious disease that is generally caused by infection with *Mycobacterium tuberculosis*, is a disease of major concern. Each year, 8 to 10 million new cases of TB are described, and, causing more than three million deaths per year, TB is a major disease in developing countries as well as an increasing problem in developed areas of the world due to, for example, antibiotic resistance.

Additionally, *M. bovis* BCG vaccination has failed to protect against TB in several trials (WHO, Tech. Rep. Ser. (1980), 651, 1-15) for reasons that are not entirely clear (Fine, Tubercle 65 (1984), 137-153). It has been shown that the vaccine strain of *M. bovis* BCG only confers protection against the severe form of miliary tuberculosis in children (Fine, Lancet 346 (1995), 1339-1345). In contrast, its protective capacity against the most common form, pulmonary tuberculosis in adults, is low and highly variable (Colditz (1994), JAMA 271, 698).

The causes for this new trend are complex: mainly, the increasing number of antibiotic applications in medicine and agriculture often combined with an improper and uncontrolled use, helps to establish resistant organisms and generate the threat of bacterial infections resistant to all available therapies.

Conventional techniques of developing antibiotics, i.e. synthesis of candidate substances and screening for antibacterial substances, even though speeded up by several orders of magnitude by the use of combinatorial approaches in recent years (e.g. US5324483, US5545568), are still too inefficient as they involve multiple screening steps of hundreds or thousands of more or less randomly chosen substances for efficiency in combating various infectious agents.

Therefore, it is a major concern to fight the growing number of bacterial infections due to an increased frequency of multiple antibiotic resistances and to improve the available antibacterial therapies.

Thus, the technical problem underlying the present invention was to provide a method and means for the development of an additional effective antibacterial therapy of infected humans and animals that can be used for the treatment of a broad spectrum of bacterial infections or diseases or disorders related to bacterial infections. The solution to this technical problem is achieved by providing the embodiments characterized in the claims.

Accordingly, the present invention relates to a method for identifying an antagonist or inhibitor of the expression of a gene encoding a polypeptide essential for bacterial growth wherein said gene is selected from the group consisting of *ygbB*, *yfhC*, *yacE*, *ychB*, *yejD*, *yrfI*, *yggJ*, *yjeE*, *yiaO*, *yrdC*, *yhbC*, *ygbP*, *ybeY*, *gcpE*, *kdtB*, *pfs*, *ycaJ*, *b1808*, *yeaA*, *yagF*, *b1983*, *yidD*, *yceG* and/or *yjbC* the sequence

of said genes being shown in Fig. 1, or a fragment or derivative or ortholog thereof, said method comprising the steps of

- (a) testing a candidate antagonist or inhibitor or a sample comprising a plurality of said candidate antagonists or inhibitors for the inhibition or reduction of transcription of said gene or a fragment or derivative thereof; or
- (b) testing a candidate antagonist or inhibitor or a sample comprising a plurality of said candidate antagonists or inhibitors for the inhibition or reduction of translation of mRNA transcribed from said gene or a fragment or derivative thereof; and
- (c) identifying an antagonist or inhibitor or a sample comprising a plurality of said candidate antagonists or inhibitors that tests positive in step (a) and/or (b).

The term "antagonist" or "inhibitor" as used herein means naturally occurring and synthetic compounds capable of counteracting or inhibiting an activity of a gene or gene product or interactions of the gene or gene product with other genes or gene products. Determining whether a compound is capable of inhibiting or counteracting specific gene expression can be done, for example, by Northern blot analysis, Western blot analysis or proteome analysis. It can further be done by monitoring the phenotypic characteristics of a bacterial cell contacted with the compounds and compare it to that of a wild-type cell. In an additional embodiment, said characteristics may be compared to that of a cell contacted with a compound which is either known to be capable or incapable of suppressing or activating the protein or gene, respectively, according to the invention. For example, the bacterial cell can be a transgenic cell and the phenotypic characteristics comprises a readout system. Further examples of determining whether a compound is capable of inhibiting or counteracting specific gene expression are described below.

The term "expression" means the production of a protein or nucleotide sequence in a cell. However, said term also includes expression of the protein in a cell-free system. It includes transcription into an RNA product, and/or translation into a polypeptide from a DNA encoding that product.

The term "transcription" as used herein means a DNA template dependent synthesis of a ribonucleic acid polymer encoding a polypeptide or a regulatory

sequence. The term "translation" as used herein means the polymerization of a polypeptide that is encoded by an RNA molecule by a protein complex.

As used in accordance with the present invention, the term "fragment or derivative" denotes any variant the amino acid or nucleotide sequence of which deviates in its primary structure, e.g., in sequence composition or in length as well as to analogue components. For example, one or more amino acids of a polypeptide may be replaced in said fragment or derivative as long as the modified polypeptides remain functionally equivalent to their described counterparts. The term "fragment or derivative" further denotes compounds analog to an antagonist or inhibitor that should have a stabilized electronic configuration and molecular conformation that allows key functional groups to be presented to the mentioned polypeptide in substantially the same way as the antagonist and inhibitor. The variant of the polypeptide may be a naturally occurring allelic variant of the polypeptide or non-naturally occurring variants of those polynucleotides.

The term "orthologs" as used herein means homologous sequences in different species that evolved from a common ancestral gene by speciation. Normally, orthologs retain the same function in the course of evolution. However, orthologous genes may or may not be responsible for a similar function (see, e.g., the glossary of the "Trends Guide to Bioinformatics", Trends Supplement 1998, Elsevier Science). Orthologous genes, nucleic acids or proteins comprise genes, nucleic acids or proteins which have one or more sequences or structural motifs in common. For example, the sequence motifs of proteins can comprise short, i.e. repetitive sequences or amino acid positions conserved in the primary structure and/or conserved in higher protein structures, e.g. secondary or tertiary structure. Orthologous nucleic acids or genes can comprise molecules having short stretches of one or more homologous (same or similar) sequences, for example protein binding boxes or structure forming boxes. Methods for the identification of a candidate ortholog of a gene or polypeptide described herein are known to those skilled in the art and are described for example in Sambrook et al. (1989), Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory Press, New York, or Ausubel (1994), Current Protocols in Mol. Biol.. The person skilled in the art knows how to identify orthologous genes, nucleic acids or polypeptides by

computer supported analysis (e.g. BLAST) of known sequences and its interpretation.

The terms "gene", "polynucleotide", "nucleic acid sequence", "nucleotide sequence", "DNA sequence" or "nucleic acid molecule" as used herein refer to polymeric forms of nucleotides of any length, either ribonucleotides or deoxyribonucleotides and only to the primary structure of the molecule. Thus, these terms include double- and single-stranded DNA, and RNA. They also include known types of modifications, for example, methylation, "caps" substitution of one or more of the naturally occurring nucleotides with an analog. Preferably, the DNA sequence of the invention comprises a coding sequence encoding at least the mature form of the above defined protein, i.e. the protein which is posttranslationally processed in its biologically active form, for example due to cleavage of leader or secretory sequences or a proprotein sequence or other natural proteolytic cleavage points.

The term "plurality of candidate antagonists or inhibitors" is to be understood as a plurality of substances which may or may not be identical.

Said antagonists or inhibitors or plurality of candidate antagonists or inhibitors may be chemically synthesized or microbiologically produced and/or comprised in, for example, samples, e.g., cell extracts from, e.g., plants, animals or microorganisms. Furthermore, said compound(s) may be known in the art but hitherto not known to be capable of suppressing or inhibiting said polypeptide. The reaction mixture may be a cell free extract or may comprise a cell or tissue culture. Suitable set ups for the method of the invention are known to the person skilled in the art and are, for example, generally described in Alberts et al., *Molecular Biology of the Cell*, third edition (1994), in particular Chapter 17. The plurality of compounds may be, e.g., added to the reaction mixture, culture medium, injected into the cell or sprayed onto the plant.

By combining computational processing of genomic information with microbial genetics, the inventors have been able to identify 24 *E. coli* essential genes and their respective orthologs (Fig. 3) that fulfill several criteria for being attractive antibacterial targets: hypothetical open reading frames, coding for essential functions (mutation is lethal for growth in rich media), broad conservation (orthologs are present in a wide range of bacteria including *H. influenza*, *S.*

pneumoniae, *H. pylori*, and *B. burgdorferi*) (Fig. 3) and low toxicity potential in higher organisms (mostly no orthologs are identified in the simple eukaryote *S. cerevisiae*). Thus, an antagonist or inhibitor of the expression of such an essential gene or of its function provides the key for an antibacterial therapy. The inventors assume that said antagonist or inhibitor stops or reduces bacterial growth and/or mediates bacterial death.

Thus, the method of the present invention provides the options of development of new broad spectrum antibiotics against new pharmaceutical important targets. The findings of the present invention are particularly important in view of the drawbacks of the present forms of treatment of bacterial infections, diseases and disorders related to bacterial infections.

In line with the above, the present invention also relates to a method for testing a candidate antagonist or inhibitor of a polypeptide or mRNA essential for bacterial growth or survival encoded by a gene selected from the group consisting of ygbB, yfhC, yacE, ychB, yejD, yrfI, yggJ, yjeE, yiaO, yrdC, yhbC, ygbP, ybeY, gcpE, kdtB, pfs, ycaJ, b1808, yeaA, yagF, b1983, yidD, yceG and/or yjbC or a fragment, derivative or ortholog thereof comprising the steps of

- (a) contacting a bacterial cell with candidate antagonist or inhibitor or a sample comprising a plurality of said candidate antagonists or inhibitors; and
- (b) testing whether said contacting leads to cell growth inhibition and/or cell death.

In a further embodiment, the present invention relates to a method for testing a candidate antagonist or inhibitor of the function of a gene essential for bacterial growth or survival wherein said gene is selected from the group consisting of ygbB, yfhC, yacE, ychB, yejD, yrfI, yggJ, yjeE, yiaO, yrdC, yhbC, ygbP, ybeY, gcpE, kdtB, pfs, ycaJ, b1808, yeaA, yagF, b1983, yidD, yceG and/or yjbC or a fragment, derivative or ortholog thereof, comprising the steps of

- (a) contacting a bacterial cell comprising said gene with a candidate antagonist or inhibitor or a sample comprising a plurality of said candidate antagonists or inhibitors; and

- (b) testing whether said contacting leads to cell growth inhibition and/or cell death.

Bacteria, for which was shown that a gene as mentioned above expressed is essential, can be used in a proliferation assay to identify both ligands and potential antagonists or inhibitors to said polypeptide encoded by said essential gene. For example, *E. coli* are grown in culture medium and incorporation of DNA precursors such as ^3H -thymidine or 5-bromo-2'-deoxyuridine (BrdU) is monitored as a parameter for DNA synthesis and cellular proliferation. Cells which have incorporated BrdU into DNA can be detected using a monoclonal antibody against BrdU and measured by an enzyme or fluorochrome-conjugated second antibody. The reaction is quantitated by fluorimetry or by spectrophotometry. The ability of the compound to be screened to inhibit proliferation may then be quantified. Further methods to determine growth and proliferation of bacteria are well known in the art, for example in Drews, Mikrobiol. Praktikum, Berlin, 1976.

Preferably, the antagonist or inhibitor binds to the gene product, i.e. the RNA or polypeptide, specifically encoded by said gene.

For example, a candidate antagonist or inhibitor not known to be capable of binding to an polypeptide encoded by a essential gene as described above can be tested to bind thereto comprising contacting a bacterial cell comprising an isolated molecule encoding said polypeptide with a candidate antagonist or inhibitor under conditions permitting binding of ligands known to bind thereto, detecting the presence of any bound ligand, and thereby determining whether such candidate antagonist or inhibitor inhibits the binding of a ligand to a polypeptide as described above.

Proteins that bind to a polypeptide as described above and might inhibit or counteract to said polypeptide can be "captured" using the yeast two-hybrid system (Fields, Nature 340 (1989), 245-246). A modified version of the yeast two-hybrid system has been described by Roger Brent and his colleagues (Gyuris, Cell 75 (1993), 791-803; Zervos, Cell 72 (1993), 223-232). Briefly, a domain of the polypeptide is used as bait for binding compounds. Positives are then selected by their ability to grow on plates lacking leucine, and then further tested for their ability to turn blue on plates with X-gal, as previously described in great detail (Gyuris, supra; WO 95/31544). Once amino acid sequences are identified which

bind to a polypeptide essential for bacterial growth or survival, these sequences can be screened for antagonist activity using, for example, the proliferation assay described above or used for screening for antagonists of said binding.

Another assay which can be performed to identify inhibitors and antagonists involves the use of combinatorial chemistry to produce random peptides which then can be screened for both binding affinity and antagonist effects. One such assay has recently been performed using random peptides expressed on the surface of a bacteriophage (Wu (1996), *Nature Biotechnology* 14, 429-431).

In a preferred embodiment of the method of the present invention said method further comprises identifying an antagonist or inhibitor optionally from said sample of candidate antagonists or inhibitors.

If a sample contains a candidate antagonist or inhibitor, or a plurality of candidate antagonists or inhibitors, as identified in the method of the invention, then it is either possible to isolate the candidate antagonists or inhibitors from the original sample identified as containing the compound capable of suppressing or inhibiting bacterial growth or survival, or one can further subdivide the original sample, for example, if it consists of a plurality of different candidate antagonists or inhibitors, so as to reduce the number of different substances per sample and repeat the method with the subdivisions of the original sample. Depending on the complexity of the samples, the steps described above can be performed several times, preferably until the sample identified according to the method of the invention only comprises a limited number of or only one substance(s). Preferably said sample comprises substances of similar chemical and/or physical properties, and most preferably said substances are identical. As regards the identification of candidate antagonists or inhibitors by any of the above-referenced embodiments of the invention, a variety of formats or tools is available to the person skilled in the art. Thus, several methods are known to the person skilled in the art for producing and screening large libraries to identify compounds having specific affinity for a target. These methods include the phage-display method in which randomized peptides are displayed from phage and screened by affinity chromatography to an immobilized receptor; see, e.g., WO 91/17271, WO 92/01047, US-A-5,223,409. In another approach, combinatorial libraries of polymers immobilized on a chip are synthesized using photolithography; see, e.g., US-A-5,143,854, WO 90/15070

and WO 92/10092. The immobilized polymers are contacted with a labeled receptor and scanned for label to identify polymers binding to the receptor. The synthesis and screening of peptide libraries on continuous cellulose membrane supports that can be used for identifying binding ligands of the polypeptide of the invention and thus possible inhibitors and antagonists is described, for example, in Kramer, *Methods Mol. Biol.* 87 (1998), 25-39. This method can also be used, for example, for determining the binding sites and the recognition motifs in the polypeptide as described above. In like manner, the substrate specificity of the DnaK chaperon was determined and the contact sites between human interleukin-6 and its receptor; see Rüdiger, *EMBO J.* 16 (1997), 1501-1507 and Weiergraber, *FEBS Lett.* 379 (1996), 122-126, respectively. Furthermore, the above-mentioned methods can be used for the construction of binding supertopes derived from the polypeptide of the invention. A similar approach was successfully described for peptide antigens of the anti-p24 (HIV-1) monoclonal antibody; see Kramer, *Cell* 91 (1997), 799-809. A general route to fingerprint analyses of peptide-antibody interactions using the clustered amino acid peptide library was described in Kramer, *Mol. Immunol.* 32 (1995), 459-465. In addition, antagonists or inhibitors of a polypeptide described above can be derived and identified from monoclonal antibodies that specifically react with said polypeptide in accordance with the methods as described in Döring, *Mol. Immunol.* 31 (1994), 1059-1067.

More recently, WO 98/25146 described further methods for screening libraries of complexes for compounds having a desired property, especially, the capacity to agonize, bind to, or antagonize a polypeptide or its cellular receptor. The complexes in such libraries comprise a compound under test, a tag recording at least one step in synthesis of the compound, and a tether susceptible to modification by a reporter molecule. Modification of the tether is used to signify that a complex contains a compound having a desired property. The tag can be decoded to reveal at least one step in the synthesis of such a compound. Other methods for identifying compounds which interact with the proteins according to the invention or nucleic acid molecules encoding such molecules are, for example, the in vitro screening with the phage display system as well as filter binding assays or "real time" measuring of interaction using, for example, the BIAcore apparatus (Pharmacia).

All these methods can be used in accordance with the present invention to identify antagonists and inhibitors of the polypeptide of the invention.

Additionally, the present invention relates in a preferred embodiment to a method comprising improving inhibitors or antagonists identified by peptidomimetics or by applying phage display or combinatorial library technique step(s). Peptidomimetics, phage display and combinatorial library techniques are well-known in the art and can be applied by the person skilled in the art without further ado to the improvement of the antagonist or inhibitor that is identified by the basic method referred to herein above.

Methods for the generation and use of peptidomimetic combinatorial libraries are described in the prior art, for example in Ostresh, *Methods In Enzymology* 267 (1996), 220-236; Dosner, *Bioorg. Med. Chem.* 4 (1996), 709-715; Beeley, *Trends Biotechn.* 12 (1994), 213-216; al-Obeidi, *Mol. Biotechn.* 9 (1998), 205-223; Wiley, *Med. Res. Rev.* 13 (1993), 327-384; Bohm, *J. Comput. Aided Mol. Des.* 10 (1996), 265-272; and Hruby, *Biopolymers* 43 (1997), 219-266.

Various sources for the basic structure of such an antagonist or inhibitor can be employed and comprise, for example, mimetic analogs of the polypeptide of the invention. Mimetic analogs of the polypeptide of the invention or biologically active fragments thereof can be generated by, for example, substituting the amino acids that are expected to be essential for the biological activity with, e.g., stereoisomers, i.e. D-amino acids; see e.g., Tsukida, *J. Med. Chem.* 40 (1997), 3534-3541. Furthermore, in case fragments are used for the design of biologically active analogs pro-mimetic components can be incorporated into a peptide to reestablish at least some of the conformational properties that may have been lost upon removal of part of the original polypeptide; see, e.g., Nachman, *Regul. Pept.* 57 (1995), 359-370. Furthermore, the polypeptide can be used to identify synthetic chemical peptide mimetics that bind to or can function as a ligand, substrate, binding partner or the receptor of the polypeptide as effectively as does the natural polypeptide; see, e.g., Engleman, *J. Clin. Invest.* 99 (1997), 2284-2292.

The structure-based design and synthesis of low-molecular-weight synthetic molecules that mimic the activity of the native biological polypeptide is further

described in, e.g., Dowd, *Nature Biotechnol.* 16 (1998), 190-195; Kieber-Emmons, *Current Opinion Biotechnol.* 8 (1997), 435-441; Moore, *Proc. West Pharmacol. Soc.* 40 (1997), 115-119; Mathews, *Proc. West Pharmacol. Soc.* 40 (1997), 121-125; Mukhija, *European J. Biochem.* 254 (1998), 433-438.

It is also well known to the person skilled in the art, that it is possible to design, synthesize and evaluate mimetics of small organic compounds that, for example, can act as a substrate or ligand to a polypeptide as encoded by the essential gene as identified above. For example, it has been described that D-glucose mimetics of hapalosin exhibited similar efficiency as hapalosin in antagonizing multidrug resistance assistance-associated protein in cytotoxicity; see Dinh, *J. Med. Chem.* 41 (1998), 981-987.

The essential gene described above or the RNA encoded thereof, as has been described above, can also serve as a target for antagonists or inhibitors. Antagonists may comprise, for example, proteins that bind to the mRNA of said gene, thereby destabilizing the native conformation of the mRNA and disturbing transcription and/or translation. Furthermore, methods are described in the literature for identifying nucleic acid molecules such as an RNA fragment that mimics the structure of a defined or undefined target RNA molecule to which a compound binds inside of a cell resulting in retardation of cell growth or cell death; see, e.g., WO 98/18947 and references cited therein. These nucleic acid molecules can be used for identifying unknown compounds of pharmaceutical and/or agricultural interest, and for identifying unknown RNA targets for use in treating a disease. These methods and compositions can be used in screening for novel antibiotics, bacteriostatics, or modifications thereof or for identifying compounds useful to alter expression levels of proteins encoded by a nucleic acid molecule. Alternatively, for example, the conformational structure of the RNA fragment which mimics the binding site can be employed in rational drug design to modify known antibiotics to make them bind more avidly to the target. One such methodology is nuclear magnetic resonance (NMR), which is useful to identify drug and RNA conformational structures. Still other methods are, for example, the drug design methods as described in WO 95/35367, US-A-5,322,933, where the crystal structure of the RNA fragment can be deduced and computer programs are utilized to design novel binding compounds which can act as antibiotics.

The candidate antagonists and inhibitors which can be tested and identified according to a method of the invention may be taken from expression libraries, e.g., cDNA expression libraries, peptides, proteins, nucleic acids, antibodies, small organic compounds, hormones, peptidomimetics, PNAs or the like (Milner, *Nature Medicine* 1 (1995), 879-880; Hupp, *Cell* 83 (1995), 237-245; Gibbs, *Cell* 79 (1994), 193-198 and references cited supra). Furthermore, genes encoding a putative regulator of an essential bacterial protein and/or which exert their effects up- or downstream said protein may be identified using, for example, insertion mutagenesis using, for example, gene targeting vectors known in the art (see, e.g., Hayashi, *Science* 258 (1992), 1350-1353; Fritze and Walden, *Gene activation by T-DNA tagging*. In *Methods in Molecular biology* 44 (Gartland, K.M.A. and Davey, M.R., eds). Totowa: Human Press (1995), 281-294) or transposon tagging (Chandlee, *Physiologia Plantarum* 78 (1990), 105-115). Said compounds can also be functional derivatives or analogues of known inhibitors or antagonists. Such useful compounds can be for example transacting factors which bind an above-described polypeptide. Identification of transacting factors can be carried out using standard methods in the art (see, e.g., Sambrook, supra, and Ausubel, supra). To determine whether a protein binds to the protein or regulatory sequence of the invention, standard native gel-shift analyses can be carried out. In order to identify a transacting factor which binds to the protein or regulatory sequence of the invention, the protein or regulatory sequence of the invention can be used as an affinity reagent in standard protein purification methods, or as a probe for screening an expression library. The identification of nucleic acid molecules which encode proteins which interact with the polypeptide described above can also be achieved, for example, as described in Scofield (*Science* 274 (1996), 2063-2065) by use of the so-called yeast "two-hybrid system"; see also the appended example. In this system, e.g., the protein encoded by the nucleic acid molecules identified in this invention or a smaller part thereof is linked to the DNA-binding domain of the GAL4 transcription factor. A yeast strain expressing this fusion gene and comprising a lacZ reporter gene driven by an appropriate promoter, which is recognized by the GAL4 or LexA transcription factor, is transformed with a library of cDNAs which will express plant genes or fragments thereof fused to an activation domain. Thus, if a peptide encoded by one of the cDNAs is able to interact with the fusion peptide comprising a peptide of a protein of

the invention, the complex is able to direct expression of the reporter gene. In this way the nucleic acid molecules and the encoded peptide can be used to identify peptides and proteins interacting with the polypeptide described above. It is apparent to the person skilled in the art that this and similar systems may then further be exploited for the identification of inhibitors or antagonists of the polypeptide.

Once the transacting factor is identified, modulation of its binding to or regulation of expression of the polypeptide described above can be pursued, beginning with, for example, screening for inhibitors against the binding of the transacting factor to the protein specified in accordance with the present invention. Inhibition of bacterial growth could then be achieved by applying the transacting factor (or its inhibitor). In addition, if the active form of the transacting factor is a dimer, dominant-negative mutants of the transacting factor could be made in order to inhibit its activity.

Thus, the present invention also relates to the use of the polypeptide as defined above for the identification of antagonists or inhibitors of a polypeptide essential for bacterial growth or survival.

In another embodiment, the present invention relates to a method for designing an improved antagonist or inhibitor for the treatment of a bacterial infection or disorder or disease related to a bacterial infection comprising the steps of

- (a) identification of the binding site of an antagonist or inhibitor to the polypeptide ygbB, yfhC, yacE, ychB, yejD, yrfI, yggJ, yjeE, yiaO, yrdC, yhbC, ygbP, ybeY, gcpE, kdtB, pfs, ycaJ, b1808, yeaA, yagF, b1983, yidD, yceG and/or yjbC, the sequence of said genes being shown in Fig. 1, or identified according to the method of the present invention, by site-directed mutagenesis and chimeric polypeptide studies;
- (b) molecular modeling of both the binding site of said antagonist or inhibitor and the structure of said polypeptide; and
- (c) modification of said antagonist or inhibitor to improve its binding specificity or affinity for the polypeptide.

Biological assays as described above or other assays such as assays based on crystallography or NMR may be employed to assess the specificity or potency of

the antagonist or inhibitor wherein the decrease of one or more activities of the polypeptide may be used to monitor said specificity or potency. All techniques employed in the various steps of the method of the invention are conventional or can be derived by the person skilled in the art from conventional techniques without further ado.

For example, identification of the binding site of said antagonist or inhibitor by site-directed mutagenesis and chimerical protein studies can be achieved by modifications in the (poly)peptide primary sequence that affect the antagonist's or inhibitor's affinity; this usually allows to precisely map the binding pocket for the drug. Identification of binding sites may be assisted by computer programs. Thus, appropriate computer programs can be used for the identification of interactive sites of a putative antagonist or inhibitor and the polypeptide of the invention by computer assisted searches for complementary structural motifs (Fassina, *Immunomethods* 5 (1994), 114-120).

As regards step (b), the following protocols may be envisaged: Once the effector site for antagonists or inhibitors has been mapped, the precise residues interacting with different parts of the antagonists or inhibitors can be identified by combination of the information obtained from mutagenesis studies (step (a)) and computer simulations of the structure of the binding site provided that the precise three-dimensional structure of the antagonists or inhibitors is known (if not, it can be predicted by computational simulation). If said antagonist or inhibitor is itself a peptide, it can be also mutated to determine which residues interact with others in the above-mentioned polypeptide essential for bacterial growth and survival.

Finally, in step (c) the antagonist or inhibitor can be modified to improve its binding affinity or its potency and specificity. If, for instance, there are electrostatic interactions between a particular residue of an polypeptide as defined above and some region of an antagonist or inhibitor molecule, the overall charge in that region can be modified to increase that particular interaction. Furthermore, the three-dimensional and/or crystallographic structure of inhibitors or antagonists of the polypeptide of the invention can be used for the design of peptidomimetic inhibitors or antagonists, e.g. in combination with said polypeptide (Rose, *Biochemistry* 35 (1996), 12933-12944; Rutenber, *Bioorg. Med. Chem.* 4 (1996), 1545-1558).

Potential antagonists/inhibitors include antisense molecules. Antisense technology can be used to control gene expression through antisense DNA or through triple-helix formation. Antisense techniques are discussed, for example, in Okano, J. *Neurochem.* 56 (1991), 560; Oligodeoxynucleotides as Antisense Inhibitors of Gene Expression, CRC Press, Boca Raton, FL (1988). Triple helix formation is discussed in, for instance, Lee, *Nucl. Acids Res.* 6 (1979), 3073; Cooney, *Science* 241 (1988), 456; and Dervan, *Science* 251 (1991), 1360. The methods are based on binding of a polynucleotide to a complementary DNA or RNA.

For example, the 5' coding portion of a polynucleotide that encodes the mature polypeptide as described above may be used to design an antisense RNA oligonucleotide of from about 10 to 40 base pairs in length. A DNA oligonucleotide is designed to be complementary to a region of the gene involved in transcription thereby preventing transcription and the production of the protein. The antisense RNA oligonucleotide hybridizes to the mRNA and blocks translation of the mRNA molecule into receptor polypeptide. As indicated, antagonist or inhibitor e.g. polyclonal and monoclonal antibody according to the teachings of the present invention can be raised according to the methods disclosed in Tartaglia, *J. Biol. Chem.* 267 (1992), 4304-4307; Tartaglia, *Cell* 73 (1993), 213-216, and PCT Application WO 94/09137.

Antibodies may be prepared by any of a variety of methods using immunogens of the polypeptide described above. As indicated, such immunogens include the full length polypeptide (which may or may not include the leader sequence) and fragments such as the ligand binding domain, the extracellular domain and the intracellular domain. These antibodies can be monoclonal antibodies, polyclonal antibodies or synthetic antibodies as well as fragments of antibodies, such as Fab^{*}, Fv, F(ab')₂, disulphide-bridged Fv or scFv fragments, etc. Monoclonal antibodies can be prepared, for example, by the techniques as originally described in Köhler and Milstein, *Nature* 256 (1975), 495, and Galfré, *Meth. Enzymol.* 73 (1981), 3, which comprise the fusion of mouse myeloma cells to spleen cells derived from immunized mammals. Furthermore, antibodies or fragments thereof to the aforementioned peptides can be obtained by using methods which are described, e.g., in Harlow and Lane "Antibodies, A Laboratory Manual", CSH Press, Cold Spring Harbor, 1988.

The antagonists or inhibitors isolated by the above methods also serve as lead compounds for the development of analog compounds. The analogs should have a stabilized electronic configuration and molecular conformation that allows key functional groups to be presented to the receptor in substantially the same way as the lead compound. In particular, the analog compounds have spatial electronic properties which are comparable to the binding region, but can be smaller molecules than the lead compound, frequently having a molecular weight below about 2 kD and preferably below about 1 kD. Identification of analog compounds can be performed through use of techniques such as self-consistent field (SCF) analysis, configuration interaction (CI) analysis, and normal mode dynamics analysis. Computer programs for implementing these techniques are available; e.g., Rein, Computer-Assisted Modeling of Receptor-Ligand Interactions (Alan Liss, New York, 1989). Methods for the preparation of chemical derivatives and analogues are well known to those skilled in the art and are described in, for example, Beilstein, Handbook of Organic Chemistry, Springer edition New York Inc., 175 Fifth Avenue, New York, N.Y. 10010 U.S.A. and Organic Synthesis, Wiley, New York, USA. Furthermore, said derivatives and analogues can be tested for their effects according to methods known in the art. Furthermore, peptidomimetics and/or computer aided design of appropriate derivatives and analogues can be used, for example, according to the methods described above.

The inhibitor or antagonist identified by the above-described method may prove useful as a pesticide, and/or antibiotic. The inhibitors and antagonists of the present invention preferably have a specificity at least substantially identical to the binding specificity of the natural ligand or binding partner of the polypeptide described above. An antagonist or inhibitor can have a binding affinity to said polypeptide of at least 10^5M^{-1} , preferably higher than 10^7M^{-1} and advantageously up to 10^{10}M^{-1} . In a preferred embodiment, an inhibitor, e.g. suppressive antibody, has an affinity of at least about 10^{-7}M , preferably at least about 10^{-9}M and most preferably at least about 10^{-11}M ; and the antagonist has an affinity of less than about 10^{-7}M , preferably less than about 10^{-9}M and most preferably in order of 10^{-11}M .

In the case of nucleic acid molecules it is preferred that they have a binding affinity to those encoding the amino acid sequences encoded in any one of SEQ ID NOS: 16 to 39 of at most 2-, 5- or 10-fold less than an exact complement of 20 consecutive nucleotides of the above described nucleic acid molecules.

In another embodiment, the present invention relates to a method for producing a therapeutic agent comprising synthesizing the above-described antagonist or inhibitor.

Preferably, the compound identified according to the above described method or its analog or derivative is further formulated in a therapeutically active form or in a form suitable for the application against bacterial infections or diseases related to such an infection. For example, it can be combined with a pharmaceutically acceptable carrier known in the art. Thus, the present invention also relates to a method of producing a (therapeutically effective) composition comprising the steps of one of the above described methods of the invention and combining the compound obtained or identified in the method of the invention or an analog or derivative thereof with a pharmaceutically acceptable carrier.

Also, the present invention relates to a composition comprising the antagonist or inhibitor mentioned above. As is evident from the above, the present invention generally relates to compositions comprising at least one of the aforementioned antagonists or inhibitors, which may be nucleic acid molecules, proteins or antibodies. Advantageously, said composition is for use as a medicament, a diagnostic means, or a kit.

The term "composition", as used in accordance with the present invention, comprises at least one small molecule or molecule as identified herein above, such as a protein, an antigenic fragment of said protein, a fusion protein, a nucleic acid molecule and/or an antibody as described above and, optionally, further molecules, either alone or in combination, like e.g. molecules which are capable of optimizing antigen processing, cytokines, immunoglobulins, lymphokines or CpG-containing DNA stretches or, optionally, adjuvants. The composition may be in solid, liquid or gaseous form and may be, inter alia, in form of (a) powder(s), (a)

tablet(s), (a) solution(s) or (an) aerosol(s). In a preferred embodiment, said composition comprises at least two, preferably three, more preferably four, most preferably five differentially synthesized proteins.

The antagonists and inhibitors of the invention appear to function against gene products which are essential in several strains or genera of bacteria. Accordingly, the above-described antagonists and inhibitors may be used to inhibit the growth of a wide spectrum of bacteria. The above described antagonists or inhibitors may be used to slow, stop, or reverse bacterial growth. Thus, the present invention also relates to a method of producing a therapeutic agent comprising the steps of the methods described hereinbefore and synthesizing the antagonist or inhibitor obtained or identified as described above or an analog or derivative thereof, preferably in an amount sufficient to provide said agent in a therapeutically effective amount to a patient.

Compounds identified by the above methods or analogs are formulated for therapeutic use as pharmaceutical compositions. The compositions can also include, depending on the formulation desired, pharmaceutically acceptable, usually sterile, non-toxic carriers or diluents, which are defined as vehicles commonly used to formulate pharmaceutical compositions for animal or human administration. The diluent is selected so as not to affect the biological activity of the combination. Examples of such diluents are distilled water, physiological saline, Ringer's solutions, dextrose solution, and Hank's solution. In addition, the pharmaceutical composition or formulation may also include other carriers, adjuvants, or nontoxic, nontherapeutic, nonimmunogenic stabilizers and the like. A therapeutically effective dose refers to that amount of protein or its antibodies, antagonists, or inhibitors which ameliorate the symptoms or condition. Therapeutic efficacy and toxicity of such compounds can be determined by standard pharmaceutical procedures in cell cultures or experimental animals, e.g., ED₅₀ (the dose therapeutically effective in 50% of the population) and LD₅₀ (the dose lethal to 50% of the population). The dose ratio between therapeutic and toxic effects is the therapeutic index, and it can be expressed as the ratio, LD₅₀/ED₅₀.

Compositions comprising such carriers can be formulated by well known conventional methods. These pharmaceutical compositions can be administered

to the subject at a suitable dose. Administration of the suitable compositions may be effected by different ways, e.g., by intravenous, intraperitoneal, subcutaneous, intramuscular, topical, intradermal, intranasal or intrabronchial administration. The dosage regimen will be determined by the attending physician and clinical factors. As is well known in the medical arts, dosages for any one patient depends upon many factors, including the patient's size, body surface area, age, the particular compound to be administered, sex, time and route of administration, general health, and other drugs being administered concurrently. Proteinaceous pharmaceutically active matter may be present in amounts between 1 ng and 10 mg per dose; however, doses below or above this exemplary range are envisioned, especially considering the aforementioned factors. Administration of the suitable compositions may be effected by different ways, e.g., by intravenous, intraperitoneal, subcutaneous, intramuscular, topical or intradermal administration. If the regimen is a continuous infusion, it should also be in the range of 1 µg to 10 mg units per kilogram of body weight per minute, respectively. Progress can be monitored by periodic assessment. The compositions of the invention may be administered locally or systemically. Administration will generally be parenterally, e.g., intravenously. The compositions of the invention may also be administered directly to the target site, e.g., by biolistic delivery to an internal or external target site or by catheter to a site in an artery. Preparations for parenteral administration include sterile aqueous or non-aqueous solutions, suspensions, and emulsions. Examples of non-aqueous solvents are propylene glycol, polyethylene glycol, vegetable oils such as olive oil, and injectable organic esters such as ethyl oleate. Aqueous carriers include water, alcoholic/aqueous solutions, emulsions or suspensions, including saline and buffered media. Parenteral vehicles include sodium chloride solution, Ringer's dextrose, dextrose and sodium chloride, lactated Ringer's, or fixed oils. Intravenous vehicles include fluid and nutrient replenishers, electrolyte replenishers (such as those based on Ringer's dextrose), and the like. Preservatives and other additives may also be present such as, for example, antimicrobials, anti-oxidants, chelating agents, and inert gases and the like. Furthermore, the pharmaceutical composition of the invention may comprise further agents such as interleukins, interferons and/or CpG-containing DNA stretches, depending on the intended use of the pharmaceutical composition.

In another embodiment, the present invention relates to a kit comprising at least one of the aforementioned antagonists or inhibitors of the invention. The kit of the invention as well as the composition may in a preferred embodiment contain further ingredients such as selection markers, antibiotics, cytokines and components for simplifying or supporting the treatment of bacterial infections or disorders or diseases related to bacterial infections. The kit of the invention may advantageously be used for carrying out the method of the invention and could be, inter alia, employed in a variety of applications referred to herein, e.g., in the diagnostic field or as research tool. The parts of the kit of the invention can be packaged individually in vials or in combination in containers or multicontainer units. Manufacture of the kit follows preferably standard procedures which are known to the person skilled in the art. The kit or its ingredients according to the invention can be used in antibacterial therapies, for example, for any of the above described methods for detecting further inhibitors and antagonists essential for bacterial growth and survival. The kit of the invention and its ingredients are expected to be very useful for the healing and protection of animals and humans suffering from a bacterial infection.

The present invention also relates to a method for treating or preventing bacterial infections or diseases or disorders related to bacterial infections comprising the step of administering to a subject in need thereof an antagonist or inhibitor identified herein above, optionally comprised in a pharmaceutical composition of the invention.

In another embodiment the present invention relates to the use of a polypeptide encoded by the gene as identified above or a fragment, derivative or ortholog thereof or of any of said genes for the identification of an antagonist or inhibitor of said polypeptide fragment, derivate or ortholog or said gene.

In a further embodiment the present invention relates to the use of said polypeptide, the therapeutic agent produced according to the invention, the antagonist or inhibitor obtained or identified by the method or use according to the invention for the preparation of a pharmaceutical composition for the treatment of

(a) bacterial infection(s), disorder(s) and/or disease(s) related to bacterial infections.

In another embodiment the present invention relates to a method for treating or preventing bacterial infections or diseases or disorders related to bacterial infections comprising the step of administering to a subject in need thereof an antagonist or inhibitor identified herein above, optionally comprised in the pharmaceutical composition according to the present invention.

In a further embodiment the present invention relates to the use of the above-described polypeptide, a fragment, derivative or ortholog thereof or of any of said genes for screening for polypeptides interacting with said polypeptide using protein-protein interaction technologies, and/or for validating such interaction as being essential for bacterial survival and/or for screening for antagonists or inhibitors of such interaction.

In a further embodiment the present invention relates to the use of the above-described polypeptide, a fragment, derivative or ortholog thereof or of any of said genes for screening of polypeptide for polypeptide binding to said polypeptide, and/or for validating the peptides binding to said polypeptide as preventing growth of bacteria or being lethal to bacteria upon expression of said polypeptides in said bacteria, and/or for screening for small molecules competitively displacing said peptides.

In another embodiment the present invention relates to the use of a conditional mutant of a gene as described above or a fragment, derivative or ortholog thereof or of surrogate ligands against said gene expressed in bacteria to induce a lethal phenotype in bacteria and/or for the analysis of said bacteria for surrogate markers by comparison of RNA or protein profiles in said bacteria with RNA or protein profiles in wild type bacteria, and/or the use of said surrogate markers for the identification of antagonists of the essential function of said gene.

In another embodiment the present invention relates to a method for identifying or isolating a surrogate marker comprising the steps as described in the above-recited method of the present invention.

In a further embodiment the present invention relates to a method for identifying or isolating a surrogate marker comprising the steps of

- (a) inducing a lethal phenotype in bacteria representing a conditional mutant of a gene selected from the group consisting of ygbB, yfhC, yacE, ychB, yejD, yrfI, yggJ, yjeE, yiaO, yrdC, yhbC, ygbP, ybeY, gcpE, kdtB, pfs, ycaJ, b1808, yeaA, yagF, b1983, yidD, yceG and/or yjbC; and
- (b) analysing said bacteria comparing the RNA or protein profile of said bacteria with wild type bacteria.

The invention also relates to the above recited genes and polypeptides and fragments, derivatives and orthologs thereof.

These and other embodiments are disclosed and encompassed by the description and examples of the present invention. Further literature concerning any one of the methods, uses and compounds to be employed in accordance with the present invention may be retrieved from public libraries, using for example electronic devices. For example the public database "Medline" may be utilized which is available on the Internet, for example under <http://www.ncbi.nlm.nih.gov/PubMed/medline.html>. Further databases and addresses, such as <http://www.ncbi.nlm.nih.gov/>, <http://www.infobiogen.fr/>, http://www.fmi.ch/biology/research_tools.html, <http://www.tigr.org/>, are known to the person skilled in the art and can also be obtained using, e.g., <http://www.lycos.com>. An overview of patent information in biotechnology and a survey of relevant sources of patent information useful for retrospective searching and for current awareness is given in Berks, TIBTECH 12 (1994), 352-364.

The present invention is further illustrated by reference to the following non-limiting examples.

Unless stated otherwise in the examples, all recombinant DNA techniques are performed according to protocols as described in Sambrook et al. (1989), *Molecular Cloning : A Laboratory Manual*. Cold Spring Harbor Laboratory Press, NY or in Volumes 1 and 2 of Ausubel et al. (1994), *Current Protocols in Molecular Biology*, Current Protocols. Standard materials and methods for plant molecular work are described in *Plant Molecular Biology Labfase* (1993) by R.D.D. Croy, jointly published by BIOS Scientific Publications Ltd. (UK) and Blackwell Scientific Publications (UK).

Brief description of the figures

- Figure 1: Sequences of the essential bacterial genes identified according to the method described in the examples
- Figure 2: PCR strategy and the position of primers used
- Figure 3: Sequence comparison table of essential *E.coli* genes with proposed orthologs from various bacteria. Unfinished genomes are indicated by asterisk. Complete genomes were analysed using BlastP2. Unfinished genomes were analysed with TBlastN. Orthologous sequences can be accessed at the respective WWW links as indicated in the footnotes.
- Figure 4: Multiple Sequence Alignment (MSA) of *E. coli* gene *ygbB* with orthologs in 5 different bacterial organisms including homology score. Similar MSA with similar results have been created for all 22 essential bacterial genes.

Example 1

An automated BLASTP-based genome comparisons to identify *E. coli* FUN genes resulted in the following list of 65 candidate genes which are conserved between *E. coli*, *B. subtilis*, *H. influenzae*, *H. pylori*, *M. tuberculosis*, *Ch. trachomatis*, *B.*

burgdorferi, *T. pallidum*, *S. pneumoniae*, *S. aureus*, *E. faecalis*, *P. aeruginosa*, *B. pertussis* and which were further analysed:

FUN Genes	Gene Bank Accession Number	FUN Genes	Gene Bank Accession Number	FUN Genes	Gene Bank Accession Number
ygbB	g1789103	yggS	g1789321	yaeE	g1786397
yhaD	g1789512	yggV	g1789324	yicC	g1790075
yhbU	g1789548	yggW	g1789325	yebK	g1788159
yhiN	g2367234	yjhG	g2367371	yhbC	g1789561
yieG	g1790150	yjiR	g1790797	ygbP	g1789104
yihZ	g1790320	yohl	g1788462	ybaX	g1786648
yjgF	g1790691	yqhThom	g1788728	yqcD	g1789158
yacE	g1786292	yfiH	g1788945	ybeY	g1786880
yaeC	g1786396	yhaR	g1789501	gcpE	g1788863
yagF	g1786464	yhdG	g1789660	kdtB	g1790065
ybeB	g1786856	yccG	g1787197	pfs	g1786354
ycfH	g147382	ychB	g1787459	sms	g1790850
ydcP	g1787705	yejD	g1788510	ycaJ	g1787119
ydiB	g1787983	yidD	g140861	yhhF	g1789875
yebi	g1788166	yrfI	g1789804	yleA	g1786882
yeeC	g1788320	yggJ	g1789315	b1808	g1788110

FUN Genes	Gene Bank Accession Number	FUN Genes	Gene Bank Accession Number	FUN Genes	Gene Bank Accession Number
yegQ	g1788397	yjeE	g1790610	yeaA	g1788077
yfcB	g1788670	yiaO	g1790004	b1675	g1787964
yfgB	g1788865	yrdC	g2367210	yhbU/yegQ ^a	g1789548 / g1788397
yfhC	g1788911	b1983	g1788294	yjgF/yhaR ^a	g1790691 / g1789501
ydiD	g1787993	yeeS	g1736671	b2385	g1788728
nlpA	g72589	yaaJ	g1786188	yicO	g1790097
yjiY	g1788997	ydhE	g1742737	yebC	g140614
ykfG	g2367100	yjcD	g396399	yohl/yhdG ^a	g1788462 / g1789660
ygcA	g1789148	yceG	g1787339	smpB	g1788973
ygfA	g1789278	yjbC	g396357		

^a: double mutants were created when the respective genes were paralogues in *E.coli*

Creating in-frame deletions of *E. coli* genes

The subsequent description of the construction of deletion mutants was carried out essentially equal for these 77 candidate genes. Particular details will exemplarily be described for one gene which gave rise to be essential (yfhC) and one which was non-essential (yggV).

1) Principle of the PCR-procedure and primer-design for *in frame* deletions:

Unless an overlapping ORF exists, primers dgenX2 and dgenX3 are designed to delete the entire ORF from ATG to STOP, e.g.: ATGgtataaatttgagtggaaggtattgcgtgTAA (SEQ ID NO: 1) (see figure). The 5'-ends of primers dgenX1 and dgenX4 contain random nucleotides followed preferably by a BamHI site (dgenX1) or a Sall site (dgenX4) for cloning into plasmid pKO3 (Link et al (1997), J Bac 179: 6228-6237). In most mutants, primers dgenX2 and dgenX3 contain a 33 bp tag sequence called "Church-tag".

Church-tag forward direction: 5'-gtataaatttgagtggaaggtattgcgtg-3' (SEQ ID NO: 2)

Church-tag reverse direction: 5'-cacgcaataaccttcacactccaaattataac-3' (SEQ ID NO: 3)

This tag is used for a subsequent PCR in which the 5'- and 3'- flanking DNA-fragments of the deletion construct are assembled.

In the few constructs lacking the "Church-tag", the primers dgenX2 and dgenX3 carry at their 5'-ends 5 random nucleotides followed by a restriction site (preferably EcoRI) which by its positioning creates the *in frame* deletion.

Oligos cgenX1 and cgenX2 are used for the verification of the chromosomal situation (wild type or deletion) after the replacement procedure (Fig. 2).

Primers for the respective candidate genes were designed as follows:

dyfhC1: 5'-GATCGGATCCAAATTCAGTTAGCCATGATGCGGTC-3'
(SEQ ID NO: 4)

dyfhC2: 5'-CACGCAATAACCTTCACACTCCAAATTTATAACCATTATA
CACGGACGCTATGC-3' (SEQ ID NO: 5)

dyfhC3: 5'-
GTTATAAATTTGGAGTGTGAAGTTATTGCGTGACGGATTAATT
TTGTTTCTCTT-3' (SEQ ID NO: 6)

dyfhC4: 5'-GATCGTCGACGCGCTCGATATCACCGATGAACAACCG-3'
(SEQ ID NO: 7)

cyfhC1: 5'-CAATCCGCTGCTTTATTTCTGTCAG-3' (SEQ ID NO: 8)

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- cyfhC2: 5'-TTATAACGAAATCAACGGGAAACCT-3' (SEQ ID NO: 9)
- dyggV1: 5'-GATCGGATCCCTCTAAAAAATAAGGAATTAAAGG-3'
(SEQ ID NO: 10)
- dyggV2: 5'-CACGCAATAACCTTCACACTCCAAATTTATAACCATAGGATAC
CTAATTAATTAAC-3' (SEQ ID NO: 11)
- dyggV3: 5'-GTTATAAATTTGGAGTGTGAAGGTTATTGCGTGAAGAGCGCC
ATTCCCAACCGT-3' (SEQ ID NO: 12)
- dyggV4: 5'-GATCGTCTGACTCATATTGCTGATAACCCGCTGCGGT-3'
(SEQ ID NO: 13)
- cyggV1: 5'-GTTGACGGCCAGGCCAACAGTCAT-3' (SEQ ID NO: 14)
- cyggV2: 5'-ATAACCCTGGGCAATCGCCTCG-3' (SEQ ID NO: 15)

Example 2

Construction of the DNA-fragments comprising the deletion

The 5'- and the 3'-flanking DNA fragments are PCR amplified in a total volume of 50 µl as follows:

Chromosomal DNA from *E. coli* strain MG1655 (100 ng/µl):

	final conc.: 1 ng/µl
10*Pwo-buffer	final conc.: 1x
dgenX1/3 (10 µM)	final conc.: 500 nM
dgenX2 (4) (10 µM)	final conc.: 500 nM
Pwo-Polymerase	final conc.: 5 U/100 µl
dNTPs (25 mM)	final conc.: 250 µM
H ₂ O	to adjust volume to 50 µl

PCR conditions:

4' 94 °C

28

30 cycles: 30" 94 °C, 30" 44 °C, 1' 72 °C

5' 72 °C

The PCR products are then purified with the High Pure PCR Purification Kit (Boehringer) to remove salts and enzyme (elute in 50 µl H₂O). Alternatively, if PCR products contain prominent impurities, the respective fragment must be purified by agarose gel extraction (Gene Clean, Dianova) before the fragment assembly.

Assembly PCR

Equal amounts of 5'- and 3'-fragment are applied as template DNA. In general a volume applied for gel electrophoresis giving an intense band is o.k. The total reaction volume is 100 µl. For the assembly the "outer" primers dgenX1 and dgenX4 were used.

5'-Fragment	approx. 10 ng
3'-Fragment	approx. 10 ng
10*Pwo-buffer	final conc.: 1x
dgenX1 (10 µM)	final conc.: 500 nM (50 pmol/100 µl)
dgenX4 (10 µM)	final conc.: 500 nM (50 pmol/100 µl)
Pwo-Pol (Boehringer)	final conc.: 5 U/100 µl
dNTPs (25mM)	final conc.: 250 µM
H ₂ O	add to 100 µl

PCR conditions:

4' 94 °C

10 cycles: 30" 94 °C, 30" 44 °C, 1' 72 °C

25 cycles: 30" 94 °C, 30" 44 °C, 3' 72 °C

5' 72 °C

The success of the PCR is checked by agarose gel electrophoresis. The assembled PCR product is purified with the High Pure PCR Purification Kit and the complete eluate of 50 µl is over-night digested with BamHI and Sall in a volume of 60 µl. After gel electrophoresis the digested product is purified with

Gene Clean (Dianova) to remove small oligonucleotides quantitatively (elution volume: 25 μ l).

Cloning into vector pKO3:

Next, the fragment is ligated into the vector pKO3 (cut with BamHI and SalI) in a 10-20 μ l reaction (T4-DNA ligase) for 2 hours at room temperature.

Transformation into DH5:

One half of the ligation mix is transformed into chemically competent *E. coli* DH5 α and clones are purified once (usually 8 clones are sufficient).

Verification of deletion constructs:

- 1) 8 clones are characterized by colony-PCR with vector pKO3-specific primers (pKO3-B1 and pKO3-S1).
- 2) Clones with the correct size of insert are double-checked by colony-PCR with gene specific primers (dgenX1 and dgenX4).

Reaction mixture for 25 μ l reaction volume:

template (colony)	1 μ l of 1 colony resuspended in 20 μ l H ₂ O
10 [*] Taq-buffer	final conc.: 1x
5 [*] Q-solution	final conc.: 1x
pKO3-B1/dgenX1 (100 μ M)	final conc.: 1 μ M (50 pmol/100 μ l)
pKO3-S1/dgenX4 (100 μ M)	final conc.: 1 μ M (50 pmol/100 μ l)
Taq-Pol (Qiagen)	final conc.: 2 U/25 μ l
dNTPs (25 mM)	final conc.: 250 μ M
H ₂ O	15.35 μ l

PCR conditions:

4' 94 °C
 25 cycles: 30" 94 °C, 30" 50 °C, 2' 65 °C
 5' 65 °C

- 3) Plasmid-DNA from 4 ml over-night culture is prepared using a QIAgen Miniprep Kit and a double restriction analysis with BamHI/Sall and EcoRI/HindIII is performed to verify the clones.

Protocol referring to the construction of assembly products by a restriction site:

The 5'- and the 3'-fragments are PCR amplified as described above. The PCR products are purified with the High Pure PCR Purification Kit (Boehringer) to remove salts and enzyme and 5 to 10 μ l are digested over night using the restriction site creating the deletion (primers 2 and 3; mostly EcoRI) in a total volume of 30 μ l. The restriction products are again purified with the High Pure PCR Purification Kit to remove nucleotides, salts and enzyme. (Alternatively: Following preparative agarose gel electrophoresis the cut fragments are isolated using Gene Clean (Dianova) and eluted in a volume of 25 μ l. The cut fragments (3-6 μ l each) are ligated in a volume of 10-15 μ l using T4-DNA ligase for 2 hours at room temperature. 5 μ l of this ligation mix is directly used as a template for a second PCR. In this PCR, the assembled fragments are amplified using primers dgenX1 and dgenX4. The reaction is set up as described above with two exceptions: 1) The total reaction volume is 100 μ l and 2) the extension step at 72 °C lasts 3'.

Example 3

The chromosomal exchange strategy

(Link et al (1997), J Bac 179: 6228-6237)

Cointegration:

Cointegration = integration of a plasmid into the chromosome by a recombination event

The pKO3 derivative is transformed into MG1655 or any recA⁺ strain

Day 1

The strain is grown at 30 °C in LB containing 20 µg/ml chloramphenicol (LB-Cam20) to an OD₆₀₀ of ~1.0. Afterwards, perform 10-fold serial dilutions in the same medium (down to 10⁻⁷). For the following plating use prewarmed LB-Cam20 agar plates. Plate 100 µl of dilutions 10⁻⁴ and 10⁻⁵ for incubation at 44 °C and 100 µl of dilutions 10⁻⁶ and 10⁻⁷ for incubation at 30 °C.

Day 2

Following incubation at the respective temperature, determine the factor c.f.u._{44 °C}/c.f.u._{30 °C} (c.f.u. = colony forming units). This factor for pKO3 without insert is in the range 1*10⁻⁴ to 5*10⁻⁴ and should be significantly larger in the case of successful cointegration. Purify 8 randomly chosen clones from the 44 °C plate twice on LB-Cam20 agar plates at 44 °C (during Day 2 and over night to Day 3). Optionally, confirm the clones for their identity as cointegrates by colony-PCR.

Resolution and counter-selection:

Resolution = resolution of the cointegrate resulting in a self replicative plasmid by a second recombination event

Counter-selection = selection against the presence of plasmid in the cell

Day 3

Pool single colonies from each of the 8 cointegrates in 100 µl LB and use this suspension as an inoculum for 10 ml LB+5 %sucrose. After growth at 30 °C (8 to 10 hours during a day is sufficient) 10-fold serial dilutions are performed and 100 µl of dilutions 10⁻⁴, 10⁻⁵, and 10⁻⁶ are plated onto LB agar+5 % sucrose and grown over night at 30 °C.

Day 4

50 single colonies are replica streaked on LB+Cam20 and LB+5 % sucrose to test for the loss of plasmid.

Example 4

Testing for essentiality of FUN genes of *E. coli* and interpretation of the results

Day 5

The clones sensitive to chloramphenicol are then tested for their genotype (wild type versus in-frame deletion) by colony-PCR using primers cgenX1 and cgenX2 (10-48 clones).

In the case of the gene *yfhC* out of 48 clones tested only wild type situation on the chromosome could be detected.

In the case of the gene *yggV* out of 48 clones 16 (= 33 %) revealed a PCR product with a size indicative for the deletion situation on the chromosome.

Are 48 clones revealing no mutant enough to claim a gene as essential? This question can be answered by asking for the number of clones that have to be tested to get a confidence of e.g. 99 % that really no mutants are present in an infinite number of clones. Provided a hypothesis H_0 means that only the wild type genotype is viable and hypothesis H_1 means that a fraction $(1-x)$ of mutants is allowed to occur together with the wild type (x) among a population of clones ($x + (1-x)$), then the probability to make the wrong decision (decision for H_0 whereas H_1 is true) can be calculated as

$$(1) \quad x^n / (1+x^n)$$

where x is the fraction of wild type clones and n is the number of clones tested.

The confidence niveau α to make the wrong decision (error probability) is given by

$$(2) \quad \alpha > x^n / (1+x^n)$$

thereby resulting in

$$(3) \quad n > \ln(\alpha / (1-\alpha)) / \ln(x)$$

for the number of clones that have to be tested to prove or disprove hypothesis H_0 .

If the average probability for obtaining wild type clones (x) in a replacement experiment is 70 % (experimentally determined for 43 non-essential genes out of 65 candidate genes), then, after testing of 26 clones which reveal a wild type genotype an uncertainty of 0.01 % error probability (α) remains that the claiming of a gene as essential could be wrong. Even if the rate of obtaining wild types (x) is set to 85 % (a value which occurs with a frequency of 10 % for replacement experiments with non-essential genes), then, by testing 32 clones (which was performed in every experiment giving rise to an essential gene) an error probability of only 0.6 % remains to chose the wrong hypothesis.

Examble 5

List of essential FUN genes obtained

By the described method the following 24 genes were obtained which gave no deletion genotype and are therefore claimed to be essential:

<i>E. coli</i>	
gene name GenBank#	
ygbB	g1789103
yfhC	g1788911
yacE	g1786292
ychB	g1787459
yejD	g1788510
yrfl	g1789804
yggJ	g1789315
yjeE	g1790610
yiaO	g1790004
yrdC	g2367210
yhbC	g1789561
ygbP	g1789104
ybeY	g1786880

gcpE	g1788863
kdtB	g1790065
pfs	g1786354
ycaJ	g1787119
b1808	g1788110
yeaA	g1788077
yagF	g1786464
b1983	g1788294
yidD	g140861
yceG	g1787339
yjbC	g396357

CLAIMS

1. A method for identifying an antagonist or inhibitor of the expression of a gene encoding a polypeptide essential for bacterial growth or survival wherein said gene is selected from the group consisting of ygbB, yfhC, yacE, ychB, yejD, yrfI, yggJ, yjeE, yiaO, yrdC, yhbC, ygbP, ybeY, gcpE, kdtB, pfs, ycaJ, b1808, yeaA, yagF, b1983, yidD, yceG and/or yjbC, the sequence of said genes being shown in Fig. 1, or a fragment, derivative or ortholog thereof, said method comprising the steps of
 - (a) testing a candidate antagonist or inhibitor or a sample comprising a plurality of said candidate antagonists or inhibitors for the inhibition or reduction of transcription of said gene or a fragment or derivative thereof; or
 - (b) testing a candidate antagonist or inhibitor or a sample comprising a plurality of said candidate antagonists or inhibitors for the inhibition or reduction of translation of mRNA transcribed from said gene or a fragment or derivative thereof; and
 - (c) identifying an antagonist or inhibitor or a sample comprising a plurality of said candidate antagonists or inhibitors that tests positive in step (a) and/or (b).
2. A method for testing a candidate antagonist or inhibitor of a polypeptide or a mRNA essential for bacterial growth or survival encoded by a gene selected from the group consisting of ygbB, yfhC, yacE, ychB, yejD, yrfI, yggJ, yjeE, yiaO, yrdC, yhbC, ygbP, ybeY, gcpE, kdtB, pfs, ycaJ, b1808, yeaA, yagF, b1983, yidD, yceG and/or yjbC, the sequence of said genes being shown in Fig. 1, or a fragment, derivative or ortholog thereof comprising the steps of
 - (a) contacting a bacterial cell with a candidate antagonist or inhibitor or a sample comprising a plurality of said candidate antagonists or inhibitors; and
 - (b) testing whether said contacting leads to cell growth inhibition and/or cell death.

3. A method for testing a candidate antagonist or inhibitor of the function of a gene essential for bacterial growth or survival wherein said gene is selected from the group consisting of ygbB, yfhC, yacE, ychB, yejD, yrfI, yggJ, yjeE, yiaO, yrdC, yhbC, ygbP, ybeY, gcpE, kdtB, pfs, ycaJ, b1808, yeaA, yagF, b1983, yidD, yceG and/or yjbC, the sequence of said genes being shown in Fig. 1, or a fragment, derivative or ortholog thereof, comprising the steps of
 - (a) contacting a bacterial cell comprising said gene with a candidate antagonist or inhibitor or a sample comprising a plurality of said candidate antagonists or inhibitors; and
 - (b) testing whether said contacting leads to cell growth inhibition and/or cell death.
4. The method of any one of claims 1 to 3 further comprising identifying an antagonist or inhibitor, optionally from said sample of candidate antagonists or inhibitors.
5. The method of any one of claims 1 to 4 wherein said inhibitor or antagonist is further improved by peptidomimetics or by applying phage display or combinatorial library technique step(s).
6. A method for designing an improved antagonist or inhibitor for the treatment of a bacterial infection or disorder or disease related to a bacterial infection comprising the steps
 - (a) identification of the binding site of an antagonist or inhibitor to the polypeptide ygbB, yfhC, yacE, ychB, yejD, yrfI, yggJ, yjeE, yiaO, yrdC, yhbC, ygbP, ybeY, gcpE, kdtB, pfs, ycaJ, b1808, yeaA, yagF, b1983, yidD, yceG and/or yjbC, the sequence of said genes being shown in Fig. 1, or obtained by or identified by the method of any one of claims 1 to 5 by site-directed mutagenesis and chimeric polypeptide studies;
 - (b) molecular modeling of both the binding site of said antagonist or inhibitor and the structure of said polypeptide; and

- (c) modification of said antagonist or inhibitor to improve its binding specificity or affinity for the polypeptide.
7. An antagonist or inhibitor of the activity of a polypeptide encoded by a gene selected from the group consisting of ygbB, yfhC, yacE, ychB, yejD, yrfI, yggJ, yjeE, yiaO, yrdC, yhbC, ygbP, ybeY, gcpE, kdtB, pfs, ycaJ, b1808, yeaA, yagF, b1983, yidD, yceG and/or yjbC, the sequence of said genes being shown in Fig. 1, or fragment, derivative or ortholog thereof or of the expression of a gene encoding said polypeptide or said fragment, derivative or ortholog or obtained by or identified by the method of any one of claims 1 to 6.
 8. A method for producing a therapeutic agent comprising synthesizing the antagonist or inhibitor identified, tested or designed according to the method of any one of claims 1 to 6 or the antagonist or inhibitor of claim 7 or an analog or derivative thereof.
 9. A method for producing a composition comprising the steps of the method of any one of claims 1 to 6 or synthesizing the antagonist or inhibitor of claim 7 and formulating said inhibitor or antagonist in a pharmaceutically acceptable form.
 10. A composition comprising an antagonist or inhibitor of claim 7, the therapeutic agent produced by the method of claim 8 or the antagonist or inhibitor obtained by or identified in the method of any one of claims 1 to 6 or produced according to claim 9 and optionally a pharmaceutically acceptable carrier.
 11. The composition of claim 10 which is a pharmaceutical composition.
 12. The composition of claim 10 which is a kit.
 13. The composition of any one of claims 10 to 12 further comprising an antibiotic and/or cytokine.

14. Use of a polypeptide encoded by a gene selected from the group consisting of ygbB, yfhC, yacE, ychB, yejD, yrfI, yggJ, yjeE, yiaO, yrdC, yhbC, ygbP, ybeY, gcpE, kdtB, pfs, ycaJ, b1808, yeaA, yagF, b1983, yidD, yceG and/or yjbC, the sequence of said genes being shown in Fig. 1, or a fragment, derivative or ortholog thereof or of any of said genes for the identification of an antagonist or inhibitor of the activity of said polypeptide or said fragment, derivative or ortholog or of the expression of a gene encoding said polypeptide or said fragment, derivative or ortholog.
15. Use of an antagonist or inhibitor of claim 7, the therapeutic agent produced by the method of claim 8 or the antagonist or inhibitor obtained by or identified in the method of any one of claims 1 to 6 or produced according to claim 9 or identified by the use of any of the claims for the preparation of a pharmaceutical composition for the treatment of (a) bacterial infection(s), disorder(s) and/or disease(s) related to bacterial infections.
16. A method for treating or preventing bacterial infections or diseases or disorders related to bacterial infections comprising the step of administering to a subject in need thereof the antagonist or inhibitor obtained by or identified in the method of any one of claims 1 to 6 or produced according to claim 9 optionally comprised in the pharmaceutical composition according to claim 11.
17. Use of a polypeptide encoded by a gene selected from the group consisting of ygbB, yfhC, yacE, ychB, yejD, yrfI, yggJ, yjeE, yiaO, yrdC, yhbC, ygbP, ybeY, gcpE, kdtB, pfs, ycaJ, b1808, yeaA, yagF, b1983, yidD, yceG and/or yjbC, the sequence of said genes being shown in Fig. 1, or a fragment, derivative or ortholog thereof or any of said genes for screening for polypeptides interacting with said polypeptide using protein-protein interaction technologies, and/or for validating such interaction as being essential for bacterial survival and/or for screening for antagonists or inhibitors of such interaction.

18. Use of a polypeptide encoded by a gene selected from the group consisting of ygbB, yfhC, yacE, ychB, yejD, yrfI, yggJ, yjeE, yiaO, yrdC, yhbC, ygbP, ybeY, gcpE, kdtB, pfs, ycaJ, b1808, yeaA, yagF, b1983, yidD, yceG and/or yjbC, the sequence of said genes being shown in Fig. 1, or a fragment, derivative or ortholog thereof or any of said genes for screening of polypeptide for polypeptide binding to said polypeptide, and/or for validating the peptides binding to said polypeptide as preventing growth of bacteria or being lethal to bacteria upon expression of said polypeptides in said bacteria, and/or for screening for small molecules competitively displacing said peptides.
19. Use of conditional mutants in a gene selected from the group consisting of ygbB, yfhC, yacE, ychB, yejD, yrfI, yggJ, yjeE, yiaO, yrdC, yhbC, ygbP, ybeY, gcpE, kdtB, pfs, ycaJ, b1808, yeaA, yagF, b1983, yidD, yceG and/or yjbC, the sequence of said genes being shown in Fig. 1, or a fragment, derivative or ortholog thereof or of surrogate ligands against said gene expressed in bacteria to induce a lethal phenotype in bacteria and/or for the analysis of said bacteria for surrogate markers by comparison of RNA or protein profiles in said bacteria with RNA or protein profiles in wild type bacteria, and/or the use of said surrogate markers for the identification of antagonists of the essential function of said gene.
20. A method for identifying or isolating a surrogate marker comprising the steps as described in claim 19.
21. A method for identifying or isolating a surrogate marker comprising the steps of
 - (a) inducing a lethal phenotype in bacteria containing a conditional mutant of a gene selected from the group consisting of ygbB, yfhC, yacE, ychB, yejD, yrfI, yggJ, yjeE, yiaO, yrdC, yhbC, ygbP, ybeY, gcpE, kdtB, pfs, ycaJ, b1808, yeaA, yagF, b1983, yidD, yceG and/or yjbC, the sequence of said genes being shown in Fig. 1; and

- (b) analysing said bacteria comparing the RNA or protein profile of said bacteria with wild type bacteria.

1) ygbB

atgcgaattggacacggtttgacgtacatgccttggcgggtgaaggcccaattatcattggtggcgctacgcattccttacgaaaa
aggattgctggcgcatctgatggcgacgtggcgctccatgcgttgaccgatgcattgcttggcgcgcgcgctgggggat
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gacgtggggaaggattgcctgtgaagcgggtggcgctactcattaaggcaacaaaatga (SEQ ID NO: 16)

2) yfhC

atgcgcgcgcgtttataacccggagtttcttctgaagtcgaatttagccacgaatactggatgcgtcacgcgctgacgctg
gcgaacagctgcctgggatgagcgggaagtgccggtcgcgcggtattagtcataacaatcgggtaatcggcgaaggctg
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gtggaaaattacggaaggaatactggcggaatgagtgccggtgctcagtgacttcttcgcatgcgcgccaggaaatga
agcgcagaaaaaagcgaatccctgcacggattaa (SEQ ID NO: 17)

3) yacE

atgaggtatagttgccttaacgggagggcattggcagtggaagagtagccgttccaatgcgttctgctgatctcggaattaacg
tcattgatgccgatattattgcgcgtcaggtggttgaaccagggtgcacctgcgctacatgccattgctgatcatttggcgctaaca
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ttgctgcagtttctcacaggaaaaaccgtaa (SEQ ID NO: 18)

4) ychB

atgcggacacagtgccctctccggcaaaaacttaactctgttttatacattaccgggtcagcgctgcggatgggtaccacacgctgc
aaacgctgtttcagtttctgattacggcgacaccatcagcattgagcttcgtgacgatggggatattcgtctgtaaaccggcttg
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aagcccgccagggtctagagcaagccccggaatggctcaatggcttggcgaaaggcgctaacttctccattgcacag
agccatgcttaa (SEQ ID NO: 19)

5) yejD

atgcgacttgataaattatcgacagcaactcggcgtagccgtgctattgccggggcgtaaatccgcggcaatcggtgcacc
gtcgatggcgaaatcgctcgtaatgcagcgttcaaaactgcttctgaacatgatgctgctacgatggcaacccgctggcgag
caacacgggtccacgttactcatgctcaataagcctcagggcgtatgttctccacggacgacccgtatccccaacgggtgctct
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aagaaattgccagcgtcgtctaa (SEQ ID NO: 20)

6) yrfI

atgattatgccgcaacatgaccaattacatcgctatctgttgaacatttgcggtgcgcgcggaactggttaacggttcggaaa
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aagaagttgatgcatcctggcggaagatggcgaaatgacatgcattgtgattactgcggttaaccactatcgttcaatgcgat
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7) yggJ

gtggggagacgacgcggttttaactatgctatccccgcattatcatcctgaaccactgaccagccattctcacatcgcgct
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ggctaa (SEQ ID NO: 22)

8) yjeE

atgatgaatcgaglaattccgctccctgatgagcaggcaacattagacctggcgagcggttagcgaagcctgcgatggc
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(SEQ ID NO: 23)

9) yiaO

atgaaattacgctctgtaacctacgcattattcattgctggcctggctgcattcagcacatcttcttggcgccacaatctttacgttt
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tcgcgacatgtttgtaaaagatgtccgcaggggagctgatctgctgaaagccgtggatgaggtgcaataa (SEQ ID NO:
24)

10) yrdC

gtgaataataacctgcaaaagagacgctatcgacgctcgatagatgttctcaatgaagaacgtgcatcgctatccaacgga
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gggctgttaaatccttcagaaatccgcgatgccctgacgggtgaactgttcgacaggggttaa (SEQ ID NO: 25)

11) yhbC

gtgggctgtccacattagagcaaaaattaacagagatgattactgcgccagttgaggccctgggtttgaactgggtggcatcg
aatatttcgcggtcgcacatccacactgcgcacatctattgatagtagaagatggcatcaatgttgatgtgctgatgagcc
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aatatccagaaggcgaacctgttccccacttttaa (SEQ ID NO: 26)

12) ygbP

atggcaaccactcatttggatgttgcgcgtggttccggcggcggaattggcgtcgaatgcaaacggaatgtcctaagcaa
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tattgcccattccatcctcagttggtcgaaggcgtgcccgaataacattaaagtcacgcgcggcgggaagattggcactggccga
gttttacctcaccgaacctccatcaggagaatacataa (SEQ ID NO: 27)

13) ybeY

atgagtcagggtatcctcgatttacaactggcatgtgaagataattccgggttaccggaagagagccagttcagacatggctg
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14) gcpE

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(SEQ ID NO: 29)

15) kdtB

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16) pfs

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17) ycaJ

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18) b1808

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19) yeaA

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ID NO: 34)

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21) b1983

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22) yidD

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23) yceG

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24) yjbC

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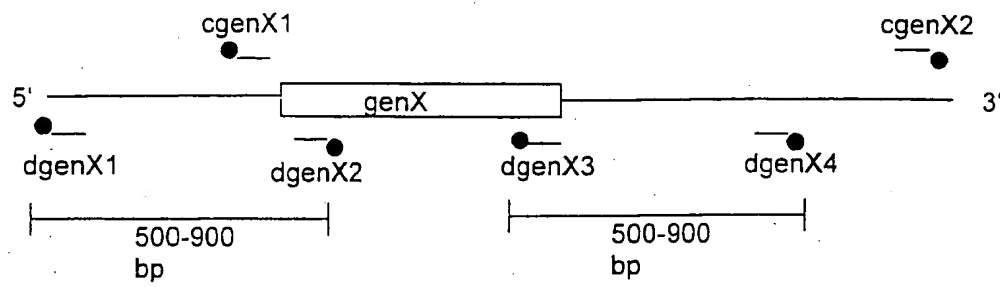


Fig. 2

<i>E. coli</i>			<i>B. subtilis</i>			<i>H. influenzae</i>			<i>H. pylori</i>			
gene name	GenBank# ¹		gene name	SWP# ² or Subtilist# ³	score	E-value	GenBank# ¹	score	E-value	GenBank# ¹	score	E-value
YgbB	g1789103		YacN	² Q06756	169	2,00E-43	1573672	205	1,00E-54	2314164	105	1,00E-24
YhbC	g1788911		YaaJ	² P21335	135	2,00E-33	1573925	175	1,00E-45	2313814	24	3
YacE	g1786292		YlaG	³ BG13824	135	2,00E-33	1573909	191	2,00E-50	2313965	87	4,00E-19
YahB	g1787459		YabH	² P37550	102	3,00E-23	1574450	317	3,00E-88	2314615	41	5,00E-05
YajD	g1788510		YizF	³ BG13940	126	6,00E-32	1574175	276	2,00E-74	2314637	61	5,00E-11
YilI	g1789804		YacC	² P37565	71	1,00E-13	1573822	286	5,00E-79	2314107	24	5,4
YggJ	g1789315		YqeU	² P54461	96	2,00E-21	1573272	312	1,00E-86	2313478	49	1,00E-07
YjeE	g1790610		YdlB	³ BG12199	89	3,00E-19	1573014	171	1,00E-44	2313840	46	9,00E-07
YiaC	g1790004		YdbE	³ BG12072	148	7,00E-37	1574060	374	1,00E-105	2314105	26	1,5
YiaC	g2367210		YwlC	² P39153	93	3,00E-19	1573655	206	3,00E-55	2313122	29	0,13
YhbC	g1789561		YlxS	² P32726	90	1,00E-19	1574740	157	2,00E-40	2314193	65	1,00E-12
YglP	g1789104		YacM	² Q06755	129	3,00E-31	1573673	233	4,00E-63	2314164	47	5,00E-07
YhcY	g1786880		YqlG	² P46347	70	8,00E-14	1572948	190	2,00E-50	2314318	54	3,00E-09
CepE	g1788863		YqlY	² P54482	318	4,00E-88	1573337	604	1,00E-174	2313753	294	3,00E-81
KdhB	g1790065		Yhl	³ BG13361	145	2,00E-36	1573650	176	5,00E-46	2314651	170	3,00E-44
pfs	g1786354		YruU	³ BG13800	244	6,00E-66	1574146	254	2,00E-69	2313168	123	4,00E-30
YcaJ	g1787119		YrvN	³ BG13808	275	5,00E-75	1574435	668	0	2314168	201	3,00E-53
b180B	g1788110		DinG	² P54394	245	6,00E-66	1573357	768	0	2313340	33	0,025
ycaA	g1788077		YppQ	² P54155	136	2,00E-32	1574293	116	3,00E-28	2313314	125	6,00E-31
yajF	g1786464		IvdD	² P51785	180	4,00E-46	1573744	168	7,00E-43	2314249	103	2,00E-23
b19B3	g1788294		YecI	³ BG12824	279	1,00E-76	1573285	161	2,00E-41	2313249	108	1,00E-25
YidD	g140861		YjaA	³ BG13865	90	6,00E-19	1176311	96	1,00E-20	2314625	40	8,00E-04

¹<http://www.ncbi.nlm.nih.gov/Entrez/protein.html>²<http://www.expasy.ch/sprot/>³<http://www.pasteur.fr/lilo/Subtilist.html>⁴<http://www.ncbi.nlm.nih.gov/BLAST/unfinishedgenomic.html>

Fig. 3

E. coli	<i>M. tuberculosis</i>			<i>Ch. trachomatis</i>			<i>B. burgdorferi</i>		
	gene name	GenBank#	score	GenBank#	score	E-value	GenBank#	score	E-value
YphB		91789103	78	3328855	62	1,00E-12	2688040	23	4,60E+00
YphC		91788911	119	3329316	120	2,00E-29	2687969	23	5,20E+00
YacE		91786292	119	3328928	62	1,00E-11	2688463	41	2,00E-05
YacB		91787459	84	3329270	82	2,00E-17	2688545	23	6,80E+00
YepD		91788510	109	3329180	105	8,00E-25	2688006	90	5,00E-20
YilL		91789804	25	3329168	23	7,80E+00	2688577	23	5,40E+00
YipJ		91789315	70	3328922	23	8,60E+00	2688252	44	4,00E-06
YieE		91790610	60	3328975	61	2,00E-11	2688077	60	1,00E-13
YhaO		91790004	27	3328868	26	1,30E+00	2688570	25	2,70E+00
YhcC		92367210	68	3328537	68	2,00E-13	2688669	73	3,00E-15
YhcG		91789561	56	3328787	26	4,00E-01	2688749	38	1,00E-04
YphP		91789104	99	3328896	95	2,00E-21	2688781	26	6,30E-01
YbeY		91786880	62	3328852	26	4,20E-01	2687941	55	6,00E-10
GapE		91788863	277	3328450	155	2,00E-39	2688019	31	5,30E-02
KdsB		91790065	140	3329163	25	9,40E-01	2688628	97	2,00E-22
pls		91786354	100	3328855	22	6,80E-01	2688208	152	8,00E-39
YcaJ		91787119	329	3328753	60	1,00E-10	2688379	55	4,00E-09
b1808		91788110	274	3329029	27	9,30E-01	2688551	30	1,30E-01
ycaA		91788077	126	3328854	27	2,20E-01	2688358	23	3,00E+00
yacF		91786464	195	3329033	28	7,30E-01	2688576	25	4,40E+00
b1983		91788294	124	3328890	118	1,00E-28	2687898	138	1,00E-34
YidD		9140861	73	3328908	56	2,00E-08	2688025	52	3,00E-07

Fig. 3 continued

<i>E. coli</i>		<i>T. pallidum</i>			<i>S. pneumoniae</i> *			<i>S. aureus</i> *		
gene name	GenBank# ¹	GenBank# ¹	score	E-value	config# ²	score	E-value	config# ²	score	E-value
YgbB	g1789103	3322804	98	1,00E-22	/	n.d.	n.d.	/	n.d.	n.d.
YhbC	g1788911	3322548	33	4,00E-03	101	71	2,00E-12	49	102	2,00E-25
YacE	g1786292	3322572	36	6,00E-04	17	109	3,00E-24	/	n.d.	n.d.
YchB	g1787459	3322649	83	7,00E-18	/	n.d.	n.d.	/	n.d.	n.d.
YcpD	g1788510	3322747	97	3,00E-22	41	166	2,00E-41	12	152	5,00E-37
Yid	g1789804	/	n.d.	n.d.	7	80	2,00E-15	249	82	9,00E-16
YggJ	g1789315	3322550	27	4,90E-01	93	65	1,00E-10	90	86	7,00E-17
Yjcd	g1790610	3323187	76	6,00E-16	140	80	2,00E-15	24	75	9,00E-14
YiaO	g1790004	3322488	28	2,90E-01	/	n.d.	n.d.	/	n.d.	n.d.
YidC	g2367210	3322447	39	6,00E-05	123	62	9,00E-10	193	76	3,00E-14
YhbC	g1789561	3322709	26	4,40E-01	47	55	8,00E-08	173	90	1,00E-18
YgbP	g1789104	3322804	58	2,00E-10	72	55	2,00E-07	/	n.d.	n.d.
YheY	g1786880	3322948	48	1,00E-07	17	60	2,00E-09	396	75	7,00E-14
GcpE	g1788863	3322731	217	3,00E-58	/	n.d.	n.d.	/	n.d.	n.d.
KatB	g1790065	3322553	100	2,00E-23	232	113	2,00E-25	205	149	2,00E-36
pls	g1786354	3322437	112	9,00E-27	156	182	5,00E-46	1235	82	1,00E-15
YcaJ	g1787119	3323329	53	1,00E-08	62	95	2,00E-23	1085	159	1,00E-38
b180B	g1788110	3322379	29	3,50E-01	114	114	5,00E-25	434	34	7,40E-01
YcaA	g1788077	3322932	111	1,00E-26	31	136	2,00E-33	422	112	1,00E-25
YagJ	g1786464	3322975	26	3,20E+00	38	202	1,00E-51	24	171	4,00E-42
b1983	g1788294	3322762	142	9,00E-36	143	360	1,00E-99	412	183	2,00E-46
YidD	g140861	Treponema F	71	4,00E-13	12	64	7,00E-11	1341	76	1,00E-14

Fig. 3 continued

<i>E. coli</i>	gene name	Genbank#	<i>E. faecalis</i> *			<i>P. aeruginosa</i> *			<i>B. pertussis</i> *		
			contig#	score	E-value	contig#	score	E-value	contig#	score	E-value
YghB		g1789103	6177	141	8,00E-34	93	181	5,00E-46	126	139	3,00E-33
YhcC		g1788911	6349	132	3,00E-31	93	151	7,00E-37	737	151	9,00E-37
YadE		g1786292	6196	111	1,00E-24	95	187	1,00E-47	924	159	3,00E-39
YehB		g1787459	6342	114	2,00E-25	95	286	2,00E-77	1062	215	9,00E-56
YefD		g1788510	6178	137	2,00E-32	94	198	8,00E-51	983	91	1,00E-18
YfhI		g1789004	6199	97	2,00E-20	97	192	4,00E-49	1085	160	2,00E-39
YggJ		g1789315	6287	75	1,00E-13	66	196	4,00E-50	551	119	4,00E-27
YjeE		g1790610	6294	29	4,00E-100	97	177	7,00E-45	762	125	4,00E-29
YjaO		g1790004	6236	125	1,00E-28	91	139	8,00E-33	459	201	1,00E-51
YjdC		g2367210	6288	96	4,00E-20	75	163	2,00E-40	362	43	4,00E-05
YhbC		g1789551	6465	103	2,00E-22	85	148	6,00E-36	371	76	4,00E-14
YghP		g1789104	6311	55	2,00E-07	93	180	2,00E-45	126	93	5,00E-19
YhcY		g1786880	6286	67	1,00E-11	91	142	3,00E-34	369	89	5,00E-18
GcpE		g1788853	/	n.d.	n.d.	91	514	1,00E-145	862	161	2,00E-39
KdsB		g1790065	6384	147	1,00E-35	84	197	1,00E-50	1097	172	2,00E-43
pls		g1786354	6495	201	1,00E-51	/	n.d.	n.d.	/	n.d.	n.d.
YadJ		g1787119	6287	138	2,00E-32	89	529	1,00E-150	1043	452	1,00E-127
YtrOB		g1788110	6265	120	7,00E-27	82	215	1,00E-55	781	255	1,00E-67
YeaA		g1788077	6315	138	3,00E-33	81	158	2,00E-39	777	146	1,00E-35
yagF		g1786464	/	n.d.	n.d.	84	169	1,00E-41	759	160	8,00E-39
Y1983		g1788294	6169	309	3,00E-84	82	145	5,00E-35	1059	155	6,00E-38
YadJ		g140861	/	n.d.	n.d.	46	76	1,00E-14	1007	74	7,00E-14

Fig. 3 continued

<i>E. coli</i>	gene name	GenBank#	<i>B. subtilis</i>	GenBank# ¹	score	E-value	<i>H. influenzae</i>	GenBank# ¹	score	E-value	<i>H. pylori</i>	GenBank# ¹	score	E-value
	yceG	g1787339		g2635201	140	2e-32		g1073838	289	2e-77		gbAAD07652.1	87	3e-16
	yjbC	g396357		g2634751	132	3e-30		g1574128	101	7e-21		g2314637	99	4e-20

<i>E. coli</i>	gene name	GenBank#	<i>M. tuberculosis</i>	GenBank# ¹	score	E-value	<i>Ch. trachomatis</i>	GenBank# ¹	score	E-value	<i>B. burgdorferi</i>	GenBank# ¹	score	E-value
	yceG	g1787339		embCAB06185	74	2e-12	/	/	n.d.	n.d.		g2688649	101	1e-20
	yjbC	g396357		g2326754	110	1e-23		g3329180	132	2e-30		g2688006	91	1e-17

<i>E. coli</i>	gene name	GenBank#	<i>T. pallidum</i>	GenBank# ¹	score	E-value	<i>S. pneumoniae</i> [*]	config# ⁴	score	E-value	<i>S. aureus</i> [*]	config# ⁴	score	E-value
	yceG	g1787339		g3322780	108	7e-23	/	/	n.d.	n.d.	/	/	n.d.	n.d.
	yjbC	g396357		g3322747	90	2e-17		12	141	5e-34		4402	134	5e-32

<i>E. coli</i>	gene name	GenBank#	<i>E. faecalis</i> [*]	config# ⁴	score	E-value	<i>P. aeruginosa</i> [*]	config# ⁴	score	E-value	<i>B. pertussis</i> [*]	config# ⁴	score	E-value
	yceG	g1787339		6216	115	4e-26		54	231	7e-61		398	209	2e-54
	yjbC	g396357		6178	118	4e-27		54	109	2e-24		190	123	9e-29

Fig. 3 continued

Multiple sequence alignment of E. coli gene ygbB with 5 orthologs from different organisms

Legend: 1 = Escherichia coli; 2 = Haemophilus influenzae;
3 = Bacillus subtilis; 4 = Synechocystis; 5 = Treponema pallidum;
6 = Helicobacter pylori; 7 = Alignment score (* identical :
chemically similar . sterically similar)

13 / 15

```

1  -----
2  -----
3  -----
4  -----
5  MRRGACVQKKEYLPLTSRQPGVCLLSEILVRALEARFFLVVTVPAGEVAYAESQVAC
6  -MSLIRVNGEAFKLSLESLEEDPFETKETLETLIKQTSVVLLAAGESRRFSQTIKKQWLR
7

```

```

1  -----
2  -----
3  -----
4  -----
5  DSRLSAFPSTRPVIYVPGAHTRSASVRAGLDAMATHAPDVVLVHDGARPFSVALIHS
6  SNHTPLWLSVYESFKEALDFKEIILVVSELDYIYIKRHYPEIKLVKGGASRQESVRNALK
7

```

Fig.

4

Fig. 4 continued

```

1  -----
2  -----
3  -----
4  -----
5  VLEATCRYGAAPVIEATDTPKGVAADGSIETHLIRSRVRLAQTPQGFCYASLCAAHHRA
6  IIDSAYTLTSDVARGLANIEALKNFLTLQQTSHYCIAPYLP CYDTAIYYNEALDREAIAK
7

```

```

1  -----
2  -----
3  -----
4  -----
5  ATDGEQYTDSELYARYG-----GTVHVCAGERSNVKITYPEDLEQRASEPALTRGISVL
6  LIQTPQLSHTKALQSALNQGFDESSAILQAFPDRVSYIEGSKDLHKLTTS GDLKHFTL
7

```

```

1  -----MRIGHGFDVHAFGGEGPIIIGGVRIPEYKGLLAHSDGDVHALTDALLGAAA
2  -----MIRIGHGFDVHAFGEDRPLIIGGVEVPYHTGFIAHSDGDVHALTDAILGAAA
3  -----MFRIGQGFVDVHQVLEGRPLIIGGIEIPEYKGLLGHSDADVLLHTVADACLGAVG
4  -----MTALRIGNGYDIHRLVGDRLIILGGVTIAHHLGLDGHSDADVLTHALMDALLGALS
5  PCTEEGALRVGLGTMHALCAGRPLILAGIHIPSKKGAQGHSDADVLAHASIDALLGAAG
6  FFNPAKDTFIGMGFDTHAFIKDKPMVLGGVVLDCEFGKLAHSDGDALLHAVIDAILGAIAK
7  : * * * * : * : : : * : * : * : * : * : * : * : * : * :

```



```

1 VFIAEDLGCHMDDVNVKATTTTEKLGFTGRGEGIACEAVALLIKATK-
2 AKIAEDLQCDIEQNVKATTTTEKLGFTGRQEGIACEAVALLIRQ---
3 KRIAEGLEADVQNVKATTTTEKLGFTGRAEGIAAQATVLIQKG---
4 ENLAKVLTIDPDLIGIKATTNERLGPTRGREGIAAYSVALLIKEG--
5 ASLAQALDTHVTRVFKAKTAERLGPVGSAAVTAQVVVLLKKI----
6 ENLSQLGLEKSQISLKATTMCKMGFIKGQEGLLVQAHSVSMRYKQKL
7   ::: *      .      :      : * * * * *      .      .      :

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SEQUENCE LISTING

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<130> D 1400 PCT

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39

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<212> DNA

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sequence

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33

<210> 3

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<212> DNA

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sequence

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33

<210> 4

<211> 36

<212> DNA

<213> Artificial Sequence

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sequence

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36

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<212> DNA

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54

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<212> DNA

<213> Artificial Sequence

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<210> 8

<211> 25

<212> DNA

<213> Artificial Sequence

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sequence

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25

<210> 9
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<212> DNA
<213> Artificial Sequence

<220>
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sequence

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25

<210> 10
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sequence

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34

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<212> DNA
<213> Artificial Sequence

<220>
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sequence

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cacgcaataa ccttcacact ccaaatttat aaccatagga tacctaatta attaac

56

<210> 12
<211> 54
<212> DNA
<213> Artificial Sequence

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<210> 13
<211> 36
<212> DNA
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36

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<212> DNA
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22

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caggcaccga agatgttgcc gcacattcca caaatgcgcg tgtttattgc cgaagatctc 360
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<211> 537
<212> DNA
<213> Escherichia coli

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ggtcgccatg atcccaccgc acatgcagaa atcatggccc tgcggcaggg tggctctggtg 240

5

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aaaactggcg ctgcccgatc tttaatggat gtgctgcac atccgggtat gaatcaccga 420
gtggaaatta cggaaggaat actggcggat gagtgccgcg cgcttgctcag tgacttcttt 480
cgcatgcgcc gccaggaaat taaagcgag aaaaaagcgc aatcctcgac ggattaa 537

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<211> 621

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<213> *Escherichia coli*

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gcgtttgctg atctcggaat taacgtcatt gatgccgata ttattgcgcg tcaggtggtt 120
gaaccagggtg cacctgcgct acatgccatt gctgatcact ttggcgctaa catgattgct 180
gctgatggaa cattgcagcg ccgggccttg cgcgagcgga tcttcgcaa cccggaagag 240
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cagcaagcta cttcccccta tgtactgtgg gttgtgccat tgctggtaga aaactcactg 360
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accatgcagc gcgatgatgt aactcgcgag catgtcgaac aaatccttgc tgctcaggca 480
acgcgcgaag ccgccttgc cgtggcagat gacgtcattg ataataacgg cgcaccggat 540
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<210> 19

<211> 852

<212> DNA

<213> *Escherichia coli*

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atcagcattg agcttcgtga cgatggggat attcgtctgt taacgcccgt tgaaggcgtg 180
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<210> 20

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<213> *Escherichia coli*

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ctgcttcctg aacatgatgt cgcttacgat ggcaaccgcg tggcgagca acacgggtcca 180
cgttacttca tgctcaataa gcctcagggc tatgtttgct ccacggacga ccctgatcac 240

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attactttct cgcgccatca ttgcgagaag acctatctgg tgacactgga atcacctgta 420
gctgacgata cggcagagca atttgctaaa ggcgtgcagc tgcataacga aaaagatctc 480
actaagcctg cgggtgctgga agtgattacc ccaacgcagg ttctgtctgac catcagcgaa 540
gggcggttatc atcaggtgaa acgcatgttc gccgccgtgg gtaaccacgt ggttgagctg 600
catcgtgaac gtattggcgg tattacgctg gatgctgatt tagcccccg tgaatatcgt 660
ccgttaactg aagaagaaat tgccagcgtc gtctaa 696

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<212> DNA

<213> Escherichia coli

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ggcgaactgg taaccgtttc ggaaaccctg caacagatcc ttgagaacca cgattatccg 120
cagcccggtta aaaacgtgct ggcagaactg ctggttgcca ccagcctggt aaccgctacg 180
ctgaagtgtt atggtgatat caccgtacag ctgcaggggc acggtccgat gaatctggcg 240
gttattaacg gtaacaataa ccagcagatg cgcggtgtgg cgcgcggtga gggcgaaatt 300
ccagaaaaatg ccgacctgaa aacgctggtc ggcaatggtt acgtggtgat caccattacc 360
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acggtttacg atccgcagga tgtggagttc aaatgcacct gctcgcgta acgttgccgc 720
gatcgctga aaacgctgcc tgatgaagaa gttgatagca tcctggcgga agatggcgaa 780
attgacatgc attgtgatta ctgcggtaac cactatctgt tcaatgcgat ggatattgct 840
gaaatccgca acaacgcgtc tccggcagat ccgcaagttc attaa 885

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<210> 22

<211> 759

<212> DNA

<213> Escherichia coli

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ctgcgcattg ggccggggca ggcgttgcaa ttgtttgacg gtagcaacca ggtctttgac 180
gccgaaatta ccagcggcag caaaaaaagc gtggaagtga aggtgctgga aggcagatc 240
gacgatcgcg aatctccgct gcatattcac ctcggtcagg tgatgtcgcg tggtagaaaa 300
atggaattta ctatccagaa atcgatcgaa ctcggtgtaa gcctcattac gccactttt 360
tctgagcgtc gcggcgtaa actggatagt gaacgtctga acaagaagct tcagcagtg 420
cagaagattg caattgctgc ctgtgagcag tgtggtcgta accgggtgcc ggaaatccgt 480
ccagcgatgg atctggaagc ctggtgtgca gagcaggatg aaggactgaa actgaatctt 540
caccgcgcgc ccagtaacag catcaatacgt ttgccgttac cggttgaacg cgtccgcctg 600
ctgattggcc cggaaggcgg ttatcggcca gatgaaattg ccatgactgc ccgctatcaa 660
tttactgata tcctgtggg acctcgctt ttgcgtacag agacaactgc gtcaccgc 720
attaccgcgc tacaagtacg atttggcgat ttgggctaa 759

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<210> 23

<211> 462

<212> DNA

<213> Escherichia coli

<400> 23

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atgatgaatc gagtaattcc gctccctgat gagcaggcaa cattagacct gggcgagcgg 60
gtagcgaag cctgcgatgg cgcaaccgta atctatctgt atggcgattt aggcgcaggt 120
aaaaccacct ttagccgggg ctttttacag gctctgggtc atcagggtaa tgcataaagc 180
cccacttata cgctggcgga accctatacg ctcgacaact taatgggtcta tcactttgat 240
ttgtaccgcc ttgccgatcc cgaggagctg gagtttatgg ggatccgcga ttattttgcc 300
aacgatgcca tctgcctggg ggagtggcca caacaaggta caggtgttct tcctgaccgc 360
gatgtcgaaa tacacattga ttatcaggca caaggccgtg aggcgcgcgt gagtgcggtt 420
tcctctgcgg gtgaattgtt gctggcgcgt ttagccggtt aa 462

```

<210> 24

<211> 987

<212> DNA

<213> Escherichia coli

<400> 24

```

atgaaattac gctctgtaac ctacgcatta ttcattgctg gcctggctgc attcagcaca 60
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catattgcgg cgaaaaaatt caatgattta ttgcaggaga gaaccaaagg cgagctgaaa 180
ttaaaactgt tcccggacag cactctcggg aacgcgcagg cgatgatcag cggcgtagct 240
ggcggcacca tcgatatgga aatgtccggc tcgaataact ttgccgggtt atcaccagt 300
atgaacttgc ttgatgtccc tttcctgttc cgcgataccg ctcacgcgca taaaacgctc 360
gacggcaaaag tcggtgatga tctgaaagcc tcacttgaag gtaaaggact gaaagtactg 420
gcctactggg aaaacggctg gcgcgatgtc accaactcgc gcgcaccggt taaaaccccc 480
gccgacctga aagggtgtaa aatccgcacc aacaatagcc cgatgaatat cggcgattc 540
aaagtctttg gcgtaaccc gatccgatg ccgtttgccg aagtctatac cgggtggaa 600
acccgcacta tcgacgtca ggaacaccgc atcaacgtcg tctggtcagc aaaaattttc 660
gaagtgcaga agttcctttc tctgacgcac cacgcctatt ccccgcttct ggtggtgatc 720
aacaagcgga agtttgatgg cttaagtcgg gagttccagc aggcgctagt ttcactcgca 780
caagaagcgg gtaactatca gcgcaaaactg gttgctgaag atcagcaaaa aatcatcgac 840
ggcatgaaag aagcgggctg ggaagtcac accgatctcg accgcaaaag ctttagcgac 900
gcactgggga atcaggttcg cgacatgttt gttaaagatg tgccgcaggg agctgatctg 960
ctgaaagccg tggatgaggt gcaataa 987

```

<210> 25

<211> 573

<212> DNA

<213> Escherichia coli

<400> 25

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gtgaataata acctgcaaag agacgtatc gcagctgcga tagatgttct caatgaagaa 60
cgtgtcatcg cctatccaac ggaagccgtt ttcggtgttg ggtgcgatcc tgatagcgaa 120
acagcagtga tgcgactgtt ggagttaaaa cagcgtccgg ttgataaggg gctgatttta 180
atcgagcaa attacgagca gcttaaacc tttattgatg acaccatgtt gactgacgtg 240
cagcgtgaaa ccattttttc ccgctggcca ggtcctgtca cctttgtctt tcccgcgct 300
gcgacaacac cgcgctggtt gacgggccgc tttgattcgc ttgctgtacg agtcaccgac 360
catccgttgg tgggtgcttt gtgccaggct tatggtaaac cgctggtttc taccagtgcc 420
aacttgagtg gattgccacc ttgtcgaaca gtagacgaag ttcgcgcaca atttgccgcg 480
gcgttcccg ttgtgcctgg tgaacgggg gggcgtttaa atccttcaga aatccgcgat 540
gcctgacgg gtgaactgtt tcgacagggg taa 573

```

<210> 26

<211> 459

<212> DNA

<213> Escherichia coli

8.

<400> 26

```

gtgggcttgt ccacattaga gcaaaaatta acagagatga ttactgcgcc agttgaggcc 60
ctgggttttg aactggttgg catcgaattt attcgcggtc gcacatccac actgcgcac 120
tatattgata gtgaagatgg catcaatggt gatgattgtg ctgatgtgag ccaccaggta 180
agtgtctgtc tggatgttga agatcccatc accgttgctt ataacctgga agtctcctca 240
ccgggtctcg atcgcccact gttcacggct gaacactacg cccgttttgt cggagaagag 300
gtgactctgg ttctccgtat ggcggtacaa aaccgtcgta aatggcaggg cgttatcaaa 360
gcggtagacg gtgaaatgat cacagttacc gtcgaaggta aagatgaagt gttcgcgctg 420
agtaatatcc agaaggcgaa cctggttccc cacttttaa 459

```

<210> 27

<211> 711

<212> DNA

<213> Escherichia coli

<400> 27

```

atggcaacca ctcatTTTgga tgtttgcgcc gtggttccgg cggccggatt tggccgtcga 60
atgcaaaccg aatgtcctaa gcaatatctc tcaatcggtg atcaaaccat tcttgaacac 120
tcgggtgcatg cgctgctggc gcatccccgg gtgaaacgtg tcgtcattgc cataagtcct 180
ggcgatagcc gttttgcaca acttctctctg gcgaatcatc cgcaaatcac cgttgtagat 240
ggcggtgatg agcgtgccga ttccgtgctg gcaggctcga aagccgctgg cgacgcgcag 300
tgggtattgg tgcattgacg cgctcgtcct tgtttgcac aggatgacct cgcgcgattg 360
ttggcgttga gcgaaaccag ccgcacgggg gggatcctcg ccgcaccagt gcgcgatact 420
atgaaacgtg ccgaaaccgg caaaaatgcc attgctcata ccgttgatcg caacggctta 480
tggcacgcgc tgacgcgcga atttttccct cgtgagctgt tacatgactg tctgacgcgc 540
gctctaaatg aaggcgcgac tattaccgac gaagcctcgg cgctggaata ttgcggttc 600
catcctcagt tggtcgaagg ccgtgcggat aacattaaag tcacgcgccc ggaagatttg 660
gcactggccg agttttacct caccgaacc atccatcagg agaatacata a 711

```

<210> 28

<211> 468

<212> DNA

<213> Escherichia coli

<400> 28

```

atgagtcagg tgatcctcga tttacaactg gcatgtgaag ataattccgg gttaccggaa 60
gagagccagt ttcagacatg gctgaatgcg gtgatccgc agtttcagga agaaccggaa 120
gtgacgattc gcgtggtcga taccgccgaa agccacagtc tgaatctgac ctatcgcggt 180
aaggataaag cgaccaacgt gctctccttc ccgtttgaag tgccgcctgg catggaaatg 240
tcgctactgg cgcattctgt tatctgccgt caggtggttg agaaggaagc tcaggagcaa 300
ggcaaaccac tggaggcgca ctgggcgcgt atggtggtgc acggcagtc gcatttgta 360
ggttacgata acatcgaaga tgacgaagca gaagaaatgg aagccctcga aacagagatt 420
atgcttgctc tgggctatga ggatccgtac attgccgaga aagaataa 468

```

<210> 29

<211> 1119

<212> DNA

<213> Escherichia coli

<400> 29

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atgcataacc aggtcccaat tcaacgtaga aaatcaacac gtatttacgt tgggaatgtg 60
ccgattggcg atggtgctcc catcgccgta cagtccatga ccaatacgcg tacgacagac 120
gtcgaagcaa cggatcaatca aatcaaggcg ctggaacgcg ttggcgctga tatcgctcgt 180
gtatccgtac cgacgatgga cgcggcagaa gcgttcaaac tcatcaaaca gcaggttaac 240
gtgccgctgg tggctgacat ccacttcgac tatcgattg cgctgaaagt agcggaaatac 300
ggcgctcgatt gctcgcgtat taaccctggc aatatcggtg atgaagagcg tattcgcatg 360

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```

gtgggtgact gtgcgcgcga taaaaacatt ccgatccgta ttggcggttaa cgccggatcg 420
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gccatgcgtc atgttgatca tctcgatcgc ctgaacttcg atcagttcaa agtcagcgtg 540
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accggcgcga acaagaaaag cggcctctat gaagatggcg tgcgcaaaag ccgtctggac 1020
aacaacgata tgatcgacca gctggaagca cgcattcgtg cgaaagccag tcagctggac 1080
gaagcgcgtc gaattgacgt tcagcaggtt gaaaaataa 1119

```

<210> 30

<211> 480

<212> DNA

<213> Escherichia coli

<400> 30

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atgcaaaaac gggcgattta tccgggtact ttcatccca ttaccaatgg tcatatcgat 60
atcgtgacgc ggcacacgca gatgttcgat cagttattc tggcgattgc cgccagcccc 120
agttaaaaaa cgatgtttac cctggaagag cgtgtggcac tggcacagca ggcaaccgcg 180
catctgggga acgtggaagt ggtcgggttt agtgatttaa tggcgaactt cgcccgtaat 240
caacacgcta cgggtgctgat tctggcctg cgtgcggtgg cagattttga atatgaaatg 300
cagctggcgc atatgaatcg ccacttaatg ccggaactgg aaagtgtgtt tctgatgccg 360
tcgaaagagt ggtcgtttat ctcttcacgc ttggtgaaag aggtggcgcg ccatacagggc 420
gatgtcaccg atttcctgcc ggagaatgct catcaggcgc tgatggcgaa gttagcgtag 480

```

<210> 31

<211> 699

<212> DNA

<213> Escherichia coli

<400> 31

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atgaaaatcg gcatcattgg tgcaatggaa gaagaagtta cgctgctgcg tgacaaaatc 60
gaaaaccgtc aaactatcag tctcggcggg tgcgaaatct ataccggcca actgaatgga 120
accgaggttg cgttcttgaa atcgggcacg ggtaaagtcg ctgcggcgct ggggtgccact 180
ttgctgttgg aacactgcaa gccagatgtg attattaaca ccggttctgc cgggtggcctg 240
gcaccaacgt tgaaagtggg cgatatcggt gtctcggacg aagcacgtta tcacgacgcg 300
gatgtcacgg catttggtta tgaatacggg cagttaccag gctgtccggc aggcctttaa 360
gctgacgata aactgatcgc tgccgctgag gcctgcattg ccgaactgaa tcttaacgct 420
gtacgtggcc tgattgttag cggcgacgct ttcatacaac gttctgttgg tctggcgaaa 480
atccgccaca acttcccaca ggccattgct gtagagatgg aagcgacggc aatcgcccat 540
gtctgccaca atttcaacgt cccgtttgtt gtcgtacgcg ccactctcca cgtggccgat 600
caacagtctc atcttagctt cgatgagttc ctggctgttg ccgctaaaca gtccagcctg 660
atggttgagt cactggtgca gaaactgca catggctaa 699

```

<210> 32

<211> 1344

<212> DNA

<213> Escherichia coli

<400> 32

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gtgagcaatc tgctgctcga tttttcggat aatacttttc aacctctggc cgcgcgatag 60
cggccagaaa atttagcaca gtatatcggc cagcaacatt tgctggctgc ggggaagccg 120

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ttgccgcgcg cctatcgaagc cgggcatttta cattctatga tcctctgggg gccgcggggt 180
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atttctgcgc tcacctctgg cgtgaaagag attcgcgagg cgatcgagcg cgccgggcaa 300
aaccgcaatg caggctgcgc cactattctt tttgttgacg aagttcaccg tttcaacaaa 360
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gccggtgagg tttacttccc gccggaaata gcacaaacac gctattattt cccgacaaac 1260
aggggccttg aaggcaagat tggcgaaaag ctgcgcctggc tggctgaaca ggatcaaaat 1320
agccccataa aacgctaccg ttaa

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<210> 33

<211> 1911

<212> DNA

<213> *Escherichia coli*

<400> 33

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gtgacggacg attttgcacc agacggtcag ctggcgaaaag cgataccagg ctttaagccg 60
cgagaaccac agcgacagat ggcggtagcc gtcacccagg cgatagaaaa aggccagccg 120
ctgggtggtgg aagcaggaac cggtagcggc ggtacacctac cttacctggc tccctgcgctg 180
cgggcgaaaa agaaagtcac tatctcgacc ggtcaaaaag cgttgcaaggc tcagctctac 240
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ctgggtgatgc gtccttacgg cgcgacgttt ctgcgcagtc tgccgcccgc gccacgcacc 1860
cgtgacattg cccgtgcggt tcgtttcctt gcgataccat cctccaggta a 1911

<210> 34
<211> 414
<212> DNA
<213> Escherichia coli

<400> 34
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gattccggct gtggctggcc cagtttctac gaaccggtaa gtgaagaatc cattcggtat 240
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<210> 35
<211> 1968
<212> DNA
<213> Escherichia coli

<400> 35
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cggcggcgga cgcacccgga cctgcacgcc cacgactttt tgccggacga caccggctg 1860
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aaaattatcg aggtaattaa cgccggtaaa aaagcgcctc gaatttaa 1968

<210> 36
 <211> 717
 <212> DNA
 <213> Escherichia coli

<400> 36
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 ccagaattaa acacatcttt aaaattcggt attgaacgtg caaagcaggc acaagttcca 180
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 atcggagcgg cagggttctgt cagctatatg tttgacaata cgggtgtgat tgtattttaa 420
 gggacagacc ctgaccatat ttttgaaatt ttacttgaag ctgaagttga tgttcgtgat 480
 gtgactgaag aagaaggtaa cattgttatt tatactgaac ctactgacct tcataaagga 540
 atcgcggctc taaaagcagc tggaatcact gagttctcaa caacagaatt agaatgatt 600
 gctcaatctg aagttgagct ttcccagaa gatttagaaa tctttgaagg gcttggtgat 660
 gcccttgaag atgacgacga tgtacaaaaa gtttatcata acgtcgcaaa tctctaa 717

<210> 37
 <211> 258
 <212> DNA
 <213> Escherichia coli

<400> 37
 atggcgccgc cactgtcgcc tggctcgagg gtccctgatag ccctcattcg ggtctatcaa 60
 cgccctgatta gtccgctact cgggcgcgat tgcctgttca ctccaacctg ttcaagctac 120
 ggaattgagg cattgcgcag gtttggagtg ataaaaggca gttggttgac ggtgaaacgc 180
 gtattaaaat gccacccttt acaccctggt ggtgacgatc ccgtcccgcc cggaccattt 240
 gataccagag aacactaa 258

<210> 38
 <211> 1023
 <212> DNA
 <213> Escherichia coli

<400> 38
 atgaaaaaag tgttattgat aatcttggtt ttgctggtgg tactgggtat cgccgctggt 60
 gtgggctgct ggaagggttc ccactctgcc gacagcaa at tgcttatcaa agaagagacg 120
 atattttacc tgaagccagg gaccggacgt ctggcgctcg gtgaacagct ttatgccgat 180
 aagatcatca atcgccacg ggtttttcaa tggctgctgc gtatcgaaac ggatctttct 240
 cactttaaag ccgggactta ccgctttaca ccgcagatga ccgtgcgca gatgctgaaa 300
 ttgctggaag gcggtaaaaga agcacagttc cctctgcgac tggtagaagg gatgcgtctg 360
 agcgattacc tcaagcaatt gcgtgaggcc ccgtatatca agcatacgt gagcgatgat 420
 aagtacgcca ccgtagcgca ggcacttgaa ctggaaaacc cggagtggat tgaagggttg 480
 ttctggccag acacctggat gtataccgcc aataccaccg atgtcgctgt actcaagcga 540
 gcgcacaaga aaatggtgaa agcggctgat agcgcctggg aagggcgtgc ggacggctctg 600
 ccttataaag ataaaaacca gttggtgacg atggcatcaa ttatcgaaaa agaaaccgcc 660
 gttgccagtg aacgcgataa ggttgctca gtatttatca accgtttacg cattggtatg 720
 cgcctgcaga ccgacccgac cgtgatttac gggatgggag agcgttataa tggcaaactt 780
 tctcgtgcag acctggaac gccgacagcg tataaacacct ataccattac cggctctgccg 840
 ccagggtcga tagcgacgcc gggggcggat tcgctgaagg ctgctgcgca tccggcaaaa 900
 acccggtatc tctattttgt ggcgatggt aaagggtggt acacgtttaa taccatctt 960
 gccagtcata acaagtctgt gcaggattat ctgaaagtc ttaaggaaaa aaatgcgcag 1020
 taa 1023

<210> 39
 <211> 873
 <212> DNA
 <213> Escherichia coli

<400> 39
 atgctgcccg actcatcagt ccgttttaaat aaatacatca gcgaaagcgg aatttgctca 60
 cgccgcgaag cggatcgcta tatcgagcaa ggcaatgtgt tccttaatgg caagcgagcc 120
 accattggcg atcaggtgaa acccggcgac gttgtgaaag taaacggtca gttgattgaa 180
 cctcggaag ccgaagattt ggtacttata gccctgaaca agcccgttgg tattgtaagc 240
 accaccgaag atggcgagcg cgataacatt gtcgatttcg ttaaccacag caaacgcgtg 300
 ttcccgattg gccgcctgga taaagactcc caggggctga ttttcctcac caatcacggc 360
 gatctggtga ataagatcct gcgtgctggc aatgatcatg agaaagagta tctggtgacg 420
 gtcgataaac cgattaccga ggagtttatt cgcggcatga gtgcgggggt gccaatcctc 480
 gggacagtga ccaaaaagtg caaagttaaa aaagaagcgc cgtttgtctt ccgcattacc 540
 ctggtgcagg ggctgaaccg tcagatccgg cgcattgtcg agcatttcgg ctatgaagtg 600
 aaaaagctgg aacgcacgcg catcatgaac gttagcttaa gcggcattcc gctgggggaa 660
 tggcgcgatt taaccgacga tgagttaatc gacctcttta agctcattga aaattcctct 720
 tccgaggtaa aacctaaagc gaaggccaaa ccgaaaacag cgggcattca acgtccagtc 780
 gttaagatgg aaaaaacggc ggaaaaaggc ggtcgccggc cgtccaacgg taagcgtttt 840
 acctcgccgg ggcgtaaaaa gaaggggcgc tga 873

<210> 40
 <211> 159
 <212> PRT
 <213> Escherichia coli

<400> 40
 Met Arg Ile Gly His Gly Phe Asp Val His Ala Phe Gly Gly Glu Gly
 1 5 10 15
 Pro Ile Ile Ile Gly Gly Val Arg Ile Pro Tyr Glu Lys Gly Leu Leu
 20 25 30
 Ala His Ser Asp Gly Asp Val Ala Leu His Ala Leu Thr Asp Ala Leu
 35 40 45
 Leu Gly Ala Ala Ala Leu Gly Asp Ile Gly Lys Leu Phe Pro Asp Thr
 50 55 60
 Asp Pro Ala Phe Lys Gly Ala Asp Ser Arg Glu Leu Leu Arg Glu Ala
 65 70 75 80
 Trp Arg Arg Ile Gln Ala Lys Gly Tyr Thr Leu Gly Asn Val Asp Val
 85 90 95
 Thr Ile Ile Ala Gln Ala Pro Lys Met Leu Pro His Ile Pro Gln Met
 100 105 110
 Arg Val Phe Ile Ala Glu Asp Leu Gly Cys His Met Asp Asp Val Asn
 115 120 125
 Val Lys Ala Thr Thr Thr Glu Lys Leu Gly Phe Thr Gly Arg Gly Glu
 130 135 140
 Gly Ile Ala Cys Glu Ala Val Ala Leu Leu Ile Lys Ala Thr Lys
 145 150 155

<210> 41
 <211> 158
 <212> PRT
 <213> Haemophilus influenzae

<400> 41
 Met Ile Arg Ile Gly His Gly Phe Asp Val His Ala Phe Gly Glu Asp
 1 5 10 15
 Arg Pro Leu Ile Ile Gly Gly Val Glu Val Pro Tyr His Thr Gly Phe
 20 25 30
 Ile Ala His Ser Asp Gly Asp Val Ala Leu His Ala Leu Thr Asp Ala
 35 40 45
 Ile Leu Gly Ala Ala Ala Leu Gly Asp Ile Gly Lys Leu Phe Pro Asp
 50 55 60
 Thr Asp Met Gln Tyr Lys Asn Ala Asp Ser Arg Gly Leu Leu Arg Glu
 65 70 75 80
 Ala Phe Arg Gln Val Gln Glu Lys Gly Tyr Lys Ile Gly Asn Val Asp
 85 90 95
 Ile Thr Ile Ile Ala Gln Ala Pro Lys Met Arg Pro His Ile Asp Ala
 100 105 110
 Met Arg Ala Lys Ile Ala Glu Asp Leu Gln Cys Asp Ile Glu Gln Val
 115 120 125
 Asn Val Lys Ala Thr Thr Thr Glu Lys Leu Gly Phe Thr Gly Arg Gln
 130 135 140
 Glu Gly Ile Ala Cys Glu Ala Val Ala Leu Leu Ile Arg Gln
 145 150 155

<210> 42
 <211> 158
 <212> PRT
 <213> Bacillus subtilis

<400> 42
 Met Phe Arg Ile Gly Gln Gly Phe Asp Val His Gln Leu Val Glu Gly
 1 5 10 15
 Arg Pro Leu Ile Ile Gly Gly Ile Glu Ile Pro Tyr Glu Lys Gly Leu
 20 25 30
 Leu Gly His Ser Asp Ala Asp Val Leu Leu His Thr Val Ala Asp Ala
 35 40 45
 Cys Leu Gly Ala Val Gly Glu Gly Asp Ile Gly Lys His Phe Pro Asp
 50 55 60
 Thr Asp Pro Glu Phe Lys Asp Ala Asp Ser Phe Lys Leu Leu Gln His
 65 70 75 80

15

Val Trp Gly Ile Val Lys Gln Lys Gly Tyr Val Leu Gly Asn Ile Asp
85 90 95

Cys Thr Ile Ile Ala Gln Lys Pro Lys Met Leu Pro Tyr Ile Glu Asp
100 105 110

Met Arg Lys Arg Ile Ala Glu Gly Leu Glu Ala Asp Val Ser Gln Val
115 120 125

Asn Val Lys Ala Thr Thr Thr Glu Lys Leu Gly Phe Thr Gly Arg Ala
130 135 140

Glu Gly Ile Ala Ala Gln Ala Thr Val Leu Ile Gln Lys Gly
145 150 155

<210> 43

<211> 161

<212> PRT

<213> Synechocystis sp.

<400> 43

Met Thr Ala Leu Arg Ile Gly Asn Gly Tyr Asp Ile His Arg Leu Val
1 5 10 15

Gly Asp Arg Pro Leu Ile Leu Gly Gly Val Thr Ile Ala His His Leu
20 25 30

Gly Leu Asp Gly His Ser Asp Ala Asp Val Leu Thr His Ala Leu Met
35 40 45

Asp Ala Leu Leu Gly Ala Leu Ser Leu Gly Asp Ile Gly His Tyr Phe
50 55 60

Pro Pro Ser Asp Ala Arg Trp Gln Gly Ala Asp Ser Leu Lys Leu Leu
65 70 75 80

Ala Gln Val His Gln Leu Ile Leu Glu Arg Gly Trp Arg Ile Asn Asn
85 90 95

Leu Asp Asn Val Ile Val Ala Glu Gln Pro Lys Leu Lys Pro His Ile
100 105 110

Gln Ala Met Lys Glu Asn Leu Ala Lys Val Leu Thr Ile Asp Pro Asp
115 120 125

Leu Ile Gly Ile Lys Ala Thr Thr Asn Glu Arg Leu Gly Pro Thr Gly
130 135 140

Arg Glu Glu Gly Ile Ala Ala Tyr Ser Val Ala Leu Leu Ile Lys Glu
145 150 155 160

Gly

<210> 44

<211> 399

<212> PRT

<213> *Treponema pallidum*

<400> 44

Met Arg Arg Gly Gly Ala Cys Val Gln Lys Lys Glu Tyr Leu Pro Leu
 1 5 10 15

Thr Ser Arg Gln Pro Gly Val Cys Leu Leu Ser Glu Ile Leu Val Arg
 20 25 30

Ala Leu Glu Ala Arg Ser Phe Phe Leu Val Val Val Thr Val Pro Ala
 35 40 45

Gly Glu Val Ala Tyr Ala Glu Ser Gln Val Ala Cys Asp Ser Arg Leu
 50 55 60

Ser Ala Phe Pro Ser Arg Thr Arg Pro Val Ile Leu Tyr Val Pro Gly
 65 70 75 80

Ala His Thr Arg Ser Ala Ser Val Arg Ala Gly Leu Asp Ala Met Ala
 85 90 95

Thr His Ala Pro Asp Val Val Leu Val His Asp Gly Ala Arg Pro Phe
 100 105 110

Val Ser Val Ala Leu Ile His Ser Val Leu Glu Ala Thr Cys Arg Tyr
 115 120 125

Gly Ala Ala Val Pro Val Ile Glu Ala Thr Asp Thr Pro Lys Gly Val
 130 135 140

Ala Ala Asp Gly Ser Ile Glu Thr His Leu Ile Arg Ser Arg Val Arg
 145 150 155 160

Leu Ala Gln Thr Pro Gln Gly Phe Cys Tyr Ala Ser Leu Cys Ala Ala
 165 170 175

His His Arg Ala Ala Thr Asp Gly Glu Gln Tyr Thr Asp Asp Ser Glu
 180 185 190

Leu Tyr Ala Arg Tyr Gly Gly Thr Val His Val Cys Ala Gly Glu Arg
 195 200 205

Ser Asn Val Lys Ile Thr Tyr Pro Glu Asp Leu Glu Gln Arg Ala Ser
 210 215 220

Glu Pro Ala Leu Thr Arg Gly Ile Ser Val Leu Pro Cys Thr Glu Glu
 225 230 235 240

Gly Ala Leu Arg Val Gly Leu Gly Thr Asp Met His Ala Leu Cys Ala
 245 250 255

Gly Arg Pro Leu Ile Leu Ala Gly Ile His Ile Pro Ser Lys Lys Gly
 260 265 270

Ala Gln Gly His Ser Asp Ala Asp Val Leu Ala His Ala Ser Ile Asp
 275 280 285

Ala Leu Leu Gly Ala Ala Gly Leu Gly Asp Ile Gly Thr Phe Phe Pro

290

295

300

Ser Cys Asp Gly Arg Trp Lys Asp Ala His Ser Cys Ala Leu Leu Arg
 305 310 315 320

His Thr Trp Gln Leu Val Arg Ala Ala Cys Trp Arg Leu Val Asn Leu
 325 330 335

Asp Ala Val Val Cys Leu Glu Gln Pro Ala Leu His Pro Phe Arg Glu
 340 345 350

Ala Met Arg Ala Ser Leu Ala Gln Ala Leu Asp Thr His Val Thr Arg
 355 360 365

Val Phe Val Lys Ala Lys Thr Ala Glu Arg Leu Gly Pro Val Gly Ser
 370 375 380

Gly Ala Ala Val Thr Ala Gln Val Val Val Leu Leu Lys Lys Ile
 385 390 395

<210> 45

<211> 406

<212> PRT

<213> Helicobacter pylori

<400> 45

Met Ser Leu Ile Arg Val Asn Gly Glu Ala Phe Lys Leu Ser Leu Glu
 1 5 10 15

Ser Leu Glu Glu Asp Pro Phe Glu Thr Lys Glu Thr Leu Glu Thr Leu
 20 25 30

Ile Lys Gln Thr Ser Val Val Leu Leu Ala Ala Gly Glu Ser Arg Arg
 35 40 45

Phe Ser Gln Thr Ile Lys Lys Gln Trp Leu Arg Ser Asn His Thr Pro
 50 55 60

Leu Trp Leu Ser Val Tyr Glu Ser Phe Lys Glu Ala Leu Asp Phe Lys
 65 70 75 80

Glu Ile Ile Leu Val Val Ser Glu Leu Asp Tyr Ile Tyr Ile Lys Arg
 85 90 95

His Tyr Pro Glu Ile Lys Leu Val Lys Gly Gly Ala Ser Arg Gln Glu
 100 105 110

Ser Val Arg Asn Ala Leu Lys Ile Ile Asp Ser Ala Tyr Thr Leu Thr
 115 120 125

Ser Asp Val Ala Arg Gly Leu Ala Asn Ile Glu Ala Leu Lys Asn Leu
 130 135 140

Phe Leu Thr Leu Gln Gln Thr Ser His Tyr Cys Ile Ala Pro Tyr Leu
 145 150 155 160

Pro Cys Tyr Asp Thr Ala Ile Tyr Tyr Asn Glu Ala Leu Asp Arg Glu
 165 170 175

Ala Ile Lys Leu Ile Gln Thr Pro Gln Leu Ser His Thr Lys Ala Leu
180
Gln Ser Ala Leu Asn Gln Gly Asp Phe Lys Asp Glu Ser Ser Ala Ile
195
Leu Gln Ala Phe Pro Asp Arg Val Ser Tyr Ile Glu Gly Ser Lys Asp
210
Leu His Lys Leu Thr Thr Ser Gly Asp Leu Lys His Phe Thr Leu Phe
225
Phe Asn Pro Ala Lys Asp Thr Phe Ile Gly Met Gly Phe Asp Thr His
245
Ala Phe Ile Lys Asp Lys Pro Met Val Leu Gly Gly Val Val Leu Asp
260
Cys Glu Phe Gly Leu Lys Ala His Ser Asp Gly Asp Ala Leu Leu His
275
Ala Val Ile Asp Ala Ile Leu Gly Ala Ile Lys Gly Asp Ile Gly
290
Glu Trp Phe Pro Asp Asn Asp Pro Lys Tyr Lys Asn Ala Ser Ser Lys
305
Glu Leu Leu Lys Ile Val Leu Asp Phe Ser Gln Ser Ile Gly Phe Glu
325
Leu Phe Glu Met Gly Ala Thr Ile Phe Ser Glu Ile Pro Lys Ile Thr
340
Pro Tyr Lys Pro Ala Ile Leu Glu Asn Leu Ser Gln Leu Leu Gly Leu
355
Glu Lys Ser Gln Ile Ser Leu Lys Ala Thr Thr Met Glu Lys Met Gly
370
Phe Ile Gly Lys Gln Glu Gly Leu Leu Val Gln Ala His Val Ser Met
385
Arg Tyr Lys Gln Lys Leu
405